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ERRATA AND AUTHORS' EMENDATIONS

Page 43, Table XVI, last figure in column headed "Number of varieties found susceptible" should be "1" instead of "21."

Page 72, line 17, "eugenol" is misspelled.

Page 74, Table I, last column, ".338" should be "33.8."

Page 76, Table III, sixth line from bottom, "Eugenol" is misspelled.

Pages 393–394, curves of Figures 2 and 3 should be interchanged.

Pages 580, 581, 582, Table I, footnote "c" applies to rows 28 and 38 only.

In footnote "d" row No. "44" should be "43." Footnote "a," page 582, should be the same as footnote "d" on preceding page.

Page 603, line 19, the word "data" should follow "climatological."

Pages 622 and 626. The graphs of Figures 1 and 3 should be interchanged, but the legends and text references are correct as given.

Page 629, line 11, "varieties of lettuce" should be "varieties of plants."

Page 686, tenth line from bottom, "nineteenth" should be "seventeenth."

Page 723. Insert in line 10 between "Sacc.," and "and" within brackets "[*Ophiobolus graminis* Sacc.]"

Page 724, seventh line from bottom, "Prenophora" should be "Pyrenophora."

Page 773, line 11, a comma and "or" should be inserted between "prematurely" and "dead."

Page 777, seventh line from bottom, "percentages" should be "percentage."

Page 789, third line from bottom, "effected" should be "affected."

Page 841, seventeenth line from bottom, "Rapd" should be "Rapp."

Page 859, lines 2 and 3, insert "spongy" before "mesophyll."

Page 862, line 2 of third paragraph from bottom, insert "spongy" before "mesophyll."

Page 917, line 9, substitute "the host" for "it."

Page 963, line 16, "are" should be "as."

Page 1001, first paragraph, last line, "days" should be "data."

Page 1005, Table III, under "Constituent," "Purine" should be "Purines."

Page 1070, second line from bottom, "II" should be "III."

Page 1071, line 28, "(I and II)" should be "(I and III)."

Page 1071, line 29, "(III)" should be "(II)."

Page 1078, eleventh line from bottom, "4.424" should be "4.425."

Page, 1191, 1193, 1195, 1197, and 1199, the running head at top of page should read: "Influence of Nitrate Nitrogen on Wheat Protein and Yield."

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FURTHER STUDIES ON THE OVERWINTERING AND DISSEMINATION OF CUCURBIT MOSAIC[1]

By S. P. DOOLITTLE, *Pathologist*, and M. N. WALKER, *Junior Pathologist*,[2] *Office of Cotton, Truck, and Forage Crop Disease Investigations, Bureau of Plant Industry, United States Department of Agriculture*

INTRODUCTION

Earlier studies of the mosaic disease of cucurbits have shown that a knowledge of the agencies by which the disease lives over winter is essential to the development of efficient control measures. The results reported in previous papers (*5*, *8*)[3] indicated that the soil was not a factor in overwintering the disease and that the seed of the cultivated cucumber, *Cucumis sativus* L., was rarely, if ever, a source of infection. There was also no evidence that the striped cucumber beetle, *Diabrotica vittata* Fabr., was an agency in the overwintering of mosaic. There was evidence, however, that the disease might overwinter on wild host plants, since it was found that seeds from mosaic plants of the wild cucumber, *Micrampelis lobata* (Michx.) Greene, would produce a certain percentage of mosaic diseased seedlings (*7*). Continued investigations during the past three years have shown that cucumber mosaic is transmissible to a number of plants of other families and have established the importance of certain wild host plants in the overwintering of the disease.

It is the purpose of this paper to present the evidence obtained from numerous experiments which have led to these conclusions.

SOIL IN RELATION TO OVERWINTERING

Experiments in Wisconsin and Michigan, prior to 1919 (*8*), indicated that cucurbit mosaic was not carried in the soil, and the results of further trials during 1919 and 1920 have strengthened this conclusion. All of these later experiments were conducted at Madison, Wis., on the same land as that used in the trials of 1917. This plat had grown cucumbers for three successive years, and the mosaic disease had been severe there each season.

EXPERIMENTS OF 1919

The plat was planted to cucumbers on June 19, using commercial seed of the Chicago Pickling variety. Ten cheesecloth cages of the

[1] Received for publication August 20, 1924; issued September, 1925.

[2] The writers are indebted to W. W. Gilbert, of the Office of Cotton, Truck, and Forage Crop Disease Investigations, for valuable suggestions and advice during the progress of this work and for assistance in obtaining certain field data and material used in these studies. They are also indebted to L. R. Jones, of the Department of Plant Pathology of the University of Wisconsin, for advice and criticism during the course of this work.

The Department of Plant Pathology, University of Wisconsin, has cooperated in furnishing land and laboratory and greenhouse facilities necessary for these investigations, and certain interested pickle companies and growers have cooperated by furnishing funds and facilities for carrying on the work on eradication and control in the field.

[3] Reference is made by number (*italic*) to "Literature cited," p. 57.

type used in the earlier experiments (*8*) were put down at the time the seed was planted and 10 more were placed over other plants on June 29. Care was taken to see that these plants were free from insects before caging and the cages were lifted only once thereafter, on July 1, in order to thin the plants. The 20 cages, covering a total of 120 plants, were removed on August 13, when the vines had become so large so as to begin to tear the cloth. The caged plants were examined and all were found to be free from mosaic. On the same date approximately 95 per cent of the 2,000 uncaged plants in the plat were mosaic-diseased.

EXPERIMENTS OF 1920

A similar experiment was conducted in 1920, using the same plat but planting a different variety of cucumbers in each row. The cages were not put down until later in the season, however, owing to the necessity of replanting most of the field on June 29. Eighteen cages were used, seven of which were put down on July 1 and the remaining eleven on July 9, when the plants were just above ground. Many of the caged plants showed injury from the striped cucumber beetle, but great care was taken to exclude these insects from the cages.

On August 2 some of the cages were lifted and the plants thinned where necessary, reducing their number from 184 to 108. At this time approximately 9 per cent of the uncaged plants in the plat were mosaic-diseased. No mosaic was found on any of the caged plants, although the cages put down on July 9 contained a number of plants affected with bacterial wilt. On August 16 the cages were finally lifted and the plants carefully examined for signs of mosaic infection. They proved healthy in all cases, while only 14 mosaic-free plants were found in a total of 2,100 plants left uncaged in the plat.

These results, combined with those earlier reported (*8*), have shown that plants protected from insects may be grown year after year on the same soil and remain absolutely free from mosaic infection in all cases, although nearly all of the uncaged plants may become infected each season. In view of these results, it seems definitely established that the soil is not an agency in overwintering cucurbit mosaic.

TRIALS OF SEED FROM MOSAIC PLANTS OF THE CULTIVATED CUCURBITS

CUCUMBER

Trials made with seed from mosaic cucumber plants prior to 1919 (*6*, *8*) indicated that mosaic infection, through the seed of the cultivated cucumber, occurred very rarely, if at all. Only one case of apparent seedling infection had occurred during trials of 10,500 plants grown from seed from mosaic cucumber fruits, but the single case was so well substantiated that it assumed a possible importance, inasmuch as the disease is transmitted by insects so rapidly that a single mosaic plant may serve as a source of infection for all the fields in the vicinity. In order to obtain more conclusive results, therefore, the tests were continued both in the field and in the greenhouse.

Field Trials of 1919

The seed used in these trials was collected by M. W. Gardner at Grass Lake, Mich., and Sparta, Wis., in the summer of 1918, from fruits which showed symptoms of the mosaic disease. The seed from each fruit was removed and planted as a separate lot. The trial plat was located at Ellison Bay, Wis., in the northern part of Door County, where mosaic was not known to occur and where cucumbers were not planted extensively. Approximately 10,000 seeds from 77 fruits were planted on June 20, but owing to the low percentage of germination, only 3,400 plants were obtained. These plants were kept under observation during the summer and inspections of the individual plants were made on July 11 and 26. Each plant was carefully examined and inoculations were made from 16 plants which showed abnormalities of growth or leaf color. None of these inoculations produced mosaic and no evidence of the disease appeared on any of the plants during the season.

Greenhouse Trials

Studies of the relation of temperature to the development of cucurbit mosaic have shown that the disease develops most rapidly at temperatures of about 30° C., and it was decided, therefore, to conduct trials of seed from mosaic plants at the temperature which seemed most favorable to the development of the disease.

Trials of 1920

Seed collected from mosaic cucumber plants at Madison in 1919 was planted during the winter in two greenhouses held at temperatures of approximately 24° and 30° C., respectively. The temperature was kept reasonably constant within limits of 2° in the house at 30° and with a variation of 5° in the other house. Plants from the seed of each fruit were grown in each of these houses, the two temperatures representing the optimum for the disease at 30° and an approximate optimum for the plant at 24°. The seeds were planted in duplicate flats of steam-sterilized soil, each flat containing 25 seeds from each of four fruits. A total of about 3,500 seedlings were obtained from the seeds of 77 fruits during the winter. The plants were kept until three leaves developed and were allowed sufficient room in the flat for normal growth. No other cucurbits were grown in the greenhouse used for these experiments and the houses were fumigated regularly in order to guard against infection by insects. No mosaic developed during these trials and the plants made normal growth in all cases.

Trials of 1921

During the winter of 1920–21, further trials were carried on in the greenhouse, using seed from the same source and growing seedlings from each fruit in duplicate series at air temperatures of approximately 30° and 24° C. Two series were conducted during the winter and early spring, a total of 2,200 plants being under observation during this time. The plants made normal growth and failed to show any symptoms of mosaic during the trials, although they were kept until the third leaf was well developed.

MUSKMELON

Greenhouse Trials of 1920–21

In addition to the trials with seed from mosaic cucumber plants, experiments were also conducted with seed from mosaic muskmelon plants, *Cucumis melo* L. As in the case of the cucumber, there had been field observations which suggested the possibility of seed transmission of the disease (*8*), but no actual experimental data had been obtained. Seeds from mosaic muskmelon plants were collected at Madison, Wis., during the summer of 1919, and an additional supply collected at Vincennes, Ind., was received from M. W. Gardner, of the Indiana Agricultural Experiment Station.

The trials were made in the greenhouse at Madison, following the same procedure as that described above in the case of the cucumber. Several series were conducted during the winters of 1920 and 1921, using seed from the same lot. The seeds were grown in duplicate series at air temperatures of 24° and 30° C. and the plants were kept under observation for approximately 30 days. Under these conditions 2,700 plants were grown until the third leaf had formed, but no symptoms of mosaic were noted on any of the plants grown at either temperature.

SQUASH AND PUMPKIN

Greenhouse Trials

Although the trials with both the cucumber and muskmelon have yielded evidence almost wholly negative in regard to seed transmission of mosaic, seeds from mosaic plants of the wild cucumber, *Micrampelis lobata*, have been shown to be carriers of the disease (*7*). In view of this peculiar circumstance, it was thought that the seeds of the larger-seeded cucurbits, such as Micrampelis, might be more likely to carry the virus than those of the smaller-seeded type, such as the cucumber and muskmelon. Experiments were therefore carried on in the greenhouses at Madison during the winter of 1921, in which seed from mosaic fruits of certain varieties of squash and pumpkin were grown in flats of sterilized soil at temperatures of 24° and 30° C., as in the earlier trials with seed from the cucumber and muskmelon.

The seed used was collected during the summer of 1920 from mosaic fruits of the Large Cheese pumpkin, *Cucurbita moschata* Duchesne, the Summer Crookneck and Golden Custard squashes, *Cucurbita pepo* L., and the Hubbard squash, *Cucurbita maxima* Duchesne. The following numbers of plants were grown from seed from the different varieties:

Summer Crookneck squash	475
Golden Custard squash	270
Hubbard squash	190
Large Cheese pumpkin	180

All of these plants were held until the third leaf had developed, and the growth, although somewhat elongated, appeared to be of normal character. No indications of mosaic were found on any of the plants of these varieties.

The accumulated evidence of all trials with seed from mosaic plants of the cultivated cucurbits has been so overwhelmingly negative that it seems evident that the seed of the cultivated varieties is

not an agency which can be considered important as a means of overwintering the mosaic disease. Only one case of apparent seedling infection has occurred in trials with approximately 22,000 cucumber plants grown from seed from mosaic plants, and none in the less extensive trials with muskmelon, squash, and pumpkin. Aside from this single instance there have been perhaps five instances of possible seedling infection from commercial cucumber seed and perhaps one in the case of the muskmelon. In such cases, however, there has usually been considerable doubt as to the actual source of infection. In any case, the rarity of such infection has been demonstrated and, in view of the prevalence of other sources of primary infection, the seed of the cultivated curcubits seems a negligible factor. It should be noted, however, as will be shown later, that the seed of the wild cucumber, *Micrampelis lobata*, is an important agency in overwintering the disease.

THE STRIPED CUCUMBER BEETLE AS A FACTOR IN OVERWINTERING MOSAIC

Studies of the striped cucumber beetle, *Diabrotica vittata*, in relation to the overwintering of mosaic, which had been in progress prior to 1919 (*8*), were continued during 1919 and 1920. The major portion of this work was in cooperation with J. E. Dudley, jr., of the Bureau of Entomology, United States Department of Agriculture. The collection and studies of the hibernation and life history of the insect were under his direction, and the studies of the beetles as possible carriers of the mosaic virus were conducted by the writers.

TRIALS WITH BEETLES FROM HIBERNATION CAGES

During the fall of 1918, several thousand beetles were collected by Dudley and placed in hibernation cages at various points. The beetles were fed on mosaic-diseased leaves and fruits of cucurbits as long as such material was available. In the latter part of April, 1919, 392 beetles which survived the winter were obtained from two of these cages and allowed to feed on healthy cucumber plants placed under cheesecloth cages in the greenhouse. Fifty small cages were placed over individual plants and a single beetle was placed in each cage. The remaining beetles were divided into lots of 25 to 80 and placed in larger cages containing several cucumber plants. In a few of the cages, young plants of *Micrampelis lobata* were included with the cucumbers. The beetles thus fed on 120 cucumber plants and 21 plants of Micrampelis. The plants were removed from the cages after an interval of four to eight days and held for at least two weeks for observation, but no signs of mosaic infection developed in either species.

EXPERIMENTS WITH BEETLES COLLECTED IN THE OPEN

At the time the beetles emerged from hibernation in the spring of 1920, a considerable number were collected in the open fields and fed on healthy cucumber plants under cages in the greenhouse. Between April 21 and May 10, 292 beetles were thus obtained and allowed to feed in cages containing 85 cucumber and 22 Micrampelis plants. No sign of mosaic appeared on any of these plants, although they were under observation for four weeks.

In addition to this 80 cucumber plants were set out on May 20 in the vicinity of cucumber fields where mosaic had occurred during the previous season. Several groups of Micrampelis plants, 110 in all, were also planted about the edge of a field where the disease seemed to occur earliest each season. All of these plants were attacked by the beetles to some extent, but none of them became infected with mosaic until late in the season.

Further trials were made during the spring of 1921 with 452 beetles collected in the neighborhood of cucumber fields between May 25 and June 4. The insects were fed on healthy cucumber plants in the same manner as in previous trials, a total of 176 plants being used in the experiments. All these plants remained healthy.

The results of these and earlier experiments (*8*) indicate that the beetle does not carry the disease through the winter, and the evidence is strengthened by experimental data which indicate that after feeding on mosaic plants they carry the virus for only a short period. The trials with beetles from hibernation cages were, however, the only ones of definite value in this regard, since it has been found that the wild cucumber, on which the insects feed after emerging from hibernation, is often affected with cucurbit mosaic. Infection obtained from beetles taken in the open, therefore, is not conclusive evidence that they carry the disease overwinter.

OVERWINTERING OF THE MOSAIC DISEASE ON WILD HOST PLANTS

Since the seed of the cultivated cucurbits seems practically eliminated as a carrier of cucurbit mosaic, and since neither the soil nor insects appear to be concerned in overwintering the disease, attention has been focused on the possible existence of wild host plants which carry the disease from year to year and serve as sources of primary infection to the cultivated cucurbits. As a result of these studies, it has been found that the wild cucumber, *Micrampelis lobata*, the milkweed, *Asclepias syriaca* L. (*9*), the poke weed, *Phytolacca decandra* L. (*10*), and probably certain *Physalis* spp., are important factors in overwintering the disease.

RELATION OF THE WILD CUCUMBER TO THE OVERWINTERING OF CUCURBIT MOSAIC

Efforts to connect the wild cucumber, *Micrampelis lobata*, with the overwintering of mosaic arose from the fact that this plant is common throughout the cucumber-growing sections of the Middle West and is affected with a mosaic disease identical in appearance with that occurring on the cultivated cucurbits. Furthermore, the wild cucumber is frequently used as an ornamental vine on fences and porches and is often found in the vicinity of cucumber fields (pl. 1, A). Observations made during 1917 and 1918 (*8*) showed that wild cucumbers growing at certain points became mosaic-diseased year after year and that the disease appeared on these plants considerably earlier than in adjacent cucumber fields. As the wild cucumber is an annual, it was thought that the disease in these cases must be carried in the seed, although there was little evidence of seed transmission in the case of the cultivated cucumber. An extensive trial of seed from mosaic Micrampelis plants was made in 1918, but, owing to unfavorable greenhouse conditions, the results were negative (*8*). A further trial during 1919 (*7*), however, gave apparent evidence of seed transmission which has been confirmed by the results of the additional tests during the last three years.

HEALTHY AND MOSAIC WILD CUCUMBER PLANTS

A. Healthy wild cucumber plants, *Micrampelis lobata*, showing typical habit of growth during flowering period. Ellison Bay, Wis. July, 1919
B. Mosaic wild cucumber plants showing mottling of foliage

TRIALS WITH SEED FROM MOSAIC PLANTS OF WILD CUCUMBER

The methods followed in the experiments with seed from mosaic plants of *Micrampelis lobata* were approximately the same in all trials. The seed was collected from plants which showed definite mosaic symptoms and, in most instances, was obtained from points where mosaic-diseased plants had appeared during previous seasons. Seed was also collected from healthy plants to serve as controls.

The seeds were dried at room temperature and then stratified in moist sand and held for a time at low temperatures. In the earlier work with the wild cucumber, considerable difficulty was encountered in obtaining germination of the seed, until, as a result of numerous experiments carried out by G. H. Harrington, formerly of the seed laboratory of the United States Department of Agriculture, it was definitely shown that a period of chilling is essential to its germination. In the trials of 1919 the seeds were stratified in a box of moist sand and allowed to remain out of doors during the winter, being brought in and planted on April 9. In the later trials the seeds were, in some cases, treated in the same way, but most of them were packed in moist sand and placed in the ice compartment of a refrigerator. Under these conditions, a period of three to five weeks was necessary for germination. A few seeds were tested for germination at room temperature at intervals of 10 days, but it was found that the seed remaining on ice would germinate as rapidly as the test samples removed to higher temperatures. When germination began, all the seeds were removed from the ice compartment and placed in moist chambers to germinate. Seeds which did not germinate within five days were again packed in sand and subjected to further chilling. Practically all the seeds which eventually germinated, however, did so within three to five days after germination was first noted, regardless of the temperature at which they were subsequently held. Little germination occurred in any of the remaining seed, although they were often kept at low temperature for six weeks thereafter.

In all trials of the seeds from mosaic Micrampelis plants, the average percentage of germination was much lower than that of the healthy control seeds which had been collected at the same time and kept under the same conditions. The mosaic seeds had an average germination of 32 per cent as compared with 67 per cent in the case of seed from healthy plants. After germination the seeds were planted in 10-inch pots of sterilized soil and the plants were grown in a greenhouse which was isolated from those in which mosaic cucumbers were present. All mosaic plants in the other houses were kept under cages in order to avoid the danger of insect transmission of the disease. No aphids or striped cucumber beetles were found on the Micrampelis plants during any of the trials, and the houses were fumigated at intervals of 10 days to guard against their presence. The results of the trials are given in Table I.

It will be noted that in all the trials a certain number of the seedlings grown from seeds of mosaic wild cucumber plants developed the mosaic disease, while none of the control plants showed signs of it at any time. All of the mosaic plants showed symptoms of the disease soon after the seed germinated. In every case the symptoms were noticeable in the first leaf, and plants which did not show signs

TABLE I.—*Results of trials with seed from mosaic and healthy plants of Micrampelis lobata*

Date of planting	Number of plants	Condition of plants used as source of seed	Number of mosaic plants	Per cent of mosaic plants	Date observed
Apr. 9, 1919	110	Mosaic	13	11	Apr. 22, 1919
Apr. 5, 1919	200	Healthy (control)	0	0	May 25, 1919
Apr. 12, 1920	174	Mosaic	10	5	Apr. 26, 1920
Apr. 15, 1920	219	Healthy (control)	0	0	May 20, 1920
May 10, 1920	211	Mosaic	21	10	May 19, 1920
May 15, 1920	150	Healthy (control)	0	0	June 8, 1920
Mar. 10, 1921	169	Mosaic	22	13	Mar. 20, 1921
Do	150	Healthy (control)	0	0	Apr. 18, 1921
Mar. 12, 1922	253	Mosaic	18	7	Mar. 29, 1922
Mar. 10, 1922	387	Healthy (control)	0	0	Apr. 21, 1922

Average infection from seed from mosaic plants, 9 per cent.

of the disease during this early period remained healthy throughout the experiment. This fact, together with the absence of mosaic on the control plants, seems to eliminate the possibility of infection from sources other than the seed.

Inoculations were made from a majority of the plants supposed to be mosaic to healthy cucumber plants and the actual presence of the disease was thus established. The early symptoms of mosaic on infected Micrampelis seedlings are often misleading, as they may frequently be confused with other abnormalities of growth which occasionally appear on plants of the wild cucumber. The first leaf of mosaic plants is usually wrinkled and somewhat mottled in appearance, but often the mottling is not sharply defined. Infected seedlings are always somewhat dwarfed, however, and the cotyledons are considerably smaller than those of healthy plants. The succeeding leaves in most cases are definitely mottled and have the curled and savoyed appearance which characterizes the mosaic disease on most cucurbits (pl. 1, B). In some instances, however, the leaves are a normal green, with the exception of minute yellow areas scattered irregularly over the surface. Such plants are somewhat dwarfed, but the leaves are regular in outline and show none of the wrinkling and curling mentioned above. In the field, where the plants have been attacked by insects or exposed to other unfavorable conditions, they often develop a similar appearance which is easily confused with mosaic. The later growth of mosaic seedlings, however, always shows typical mosaic symptoms.

The consistent appearance of mosaic on a portion of the seedlings grown from seeds of mosaic Micrampelis, together with the fact that the disease did not develop on the remaining plants during their later growth or on plants grown from healthy seed, seems to warrant the conclusion that the seed of the wild cucumber acts as a carrier of the disease. This has been further substantiated by the field observations described below.

DEVELOPMENT OF MOSAIC ON WILD CUCUMBER SEEDLINGS IN THE FIELD

Before definite evidence of seed transmission of Micrampelis mosaic was obtained it was already known that the disease was of common occurrence on this host (*8*) and that mosaic plants could be found in the spring before cultivated cucurbits had been planted. Since

this latter fact confirmed the results of the seed trials, a series of observations was made each year at points where mosaic Micrampelis plants had originally been noted.

CAGING EXPERIMENTS

Early in the spring of 1919 large cheesecloth cages were constructed over two spots where mosaic wild cucumbers had been found during 1917 and 1918. The cages covered a space of 4 by 6 feet and were 3 feet high, with the lower edge sunk 6 inches in the ground. The first cage was put down on April 10 before any of the Micrampelis seed had germinated, and the second was set out on May 1 over seedlings that had just appeared. No striped beetles or other cucumber insects had appeared at this time and the seedlings showed no evidence of insect injury. The cages were watched carefully in order to repair any breaks in the cloth and, as far as could be determined, no insects gained entrance during the experiment. On May 28 the plants in the cages were examined, and in the first cage 2 out of 11 plants showed definite mosaic symptoms. In the cage put down on May 1, 4 out of 26 plants were mosaic diseased. No insects were found in the cage and the plants showed no insect injury. It having been shown that the soil is not a carrier of the disease, these results offered further indication that the seed of Micrampelis acts as a carrier of cucurbit mosaic.

FIELD OBSERVATIONS

Various centers of mosaic wild cucumber plants found between 1917 and 1919 have since been kept under observation in order to determine the exact period at which the disease appears in the spring. These observations, begun in 1918 (*8*), have given abundant evidence that the disease develops in the same spot each season and that its appearance may be noted on the plants while in the seedling stages. The results, as shown in Table II, include observations at 12 of these points, in all of which the disease was found during successive seasons.

TABLE II.—*Record of appearance of mosaic on seedlings of Micrampelis lobata at same points during successive seasons*

Plant group	Locality	Period of observations					
		May 1 to June 1, 1919		May 1 to June 1, 1920		May 1 to June 1, 1921	
		Number of plants	Number of mosaic plants	Number of plants	Number of mosaic plants	Number of plants	Number of mosaic plants
[a] I	Madison, Wis.	68	9	12	2	0	----------
II	do.	90	15	41	9	49	6
[a] III	do.	108	9	72	11	32	7
[a] IV	do.	40	7	30	4	33	6
V	do.	15	3	15	2	34	5
VI	do.	29	3	21	5	39	5
VII	do.	----------	[b]	625	60	300	18
VIII	do.	----------	[b]	28	3	76	9
IX	Portage, Wis.	----------	[b]	350	25	250	34
X	Marengo, Ill.	----------	----------	125	8	89	11
XI	do.	----------	----------	35	7	19	4
XII	do.	----------	----------	10	2	28	4
	Per cent of plants mosaic	----------	13	----------	10	----------	11

[a] Mosaic plants also found during May, 1918. [b] Mosaic plants found during July and August.

The wild cucumber seedlings in the vicinity of Madison, Wis., usually appear in the field between May 1 and May 15, and practically all the observations were made within two weeks after the development of the first leaf. All the plants which developed mosaic showed definite symptoms of the disease in the first leaves, and in many cases the presence of the disease was confirmed by inoculations from suspected plants to healthy cucumbers in the greenhouse. The possibility of infection from other sources was slight, as cultivated cucurbits are rarely planted at the time these observations were made, and near-by groups of healthy Micrampelis plants remained mosaic-free each season. The experiments previously mentioned, in which mosaic Micrampelis plants appeared in spots which had been caged early in the spring, also seem to reduce the possibility of the infection coming from any source except the seed. As will be shown later in this paper, the milkweed, *Asclepias syriaca*, is also an agency in overwintering cucurbit mosaic, but as the mosaic milkweed shoots do not appear until some time after the disease is found on the wild cucumber seedlings, the infection on Micrampelis could hardly come from this source. It is also of interest to note that the percentage of mosaic plants found in the open each year is approximately the same as that obtained in trials with seed from mosaic wild cucumber plants in the greenhouse. All the evidence, therefore, seems to warrant the conclusion that cucurbit mosaic is carried in the seed of mosaic plants of the wild cucumber.

It is a peculiar fact that cucurbit mosaic should be carried in the seed of the wild cucumber but not in that of the cultivated cucurbits. This anomaly is as yet unexplained, but Butler (*3*), in a review of the literature on mosaic diseases, makes an interesting suggestion. He points out that the embryo is not connected with the vascular system, as the bundles do not penetrate the nucellus, and states that—

> In the Cucurbitaceae the nucellus is provided on the outside of its epidermis with a well-marked cuticle, and this epidermis persists in the ripe seed, though the rest of the nucellus is absorbed. The stalk end of the ovule (the chalaza) is suberified. Thus the whole of the central part of the ovule is cut off from the seed coats and stalk by cutin or suberin. The embryo also, in the spherical stage, is provided with a complete investment of cuticle over its free parts. All the evidence available in regard to infection by the virus diseases indicates strongly that they are unable to pass through cutin and probably unable to pass through suberin (e. g., uninjured branches or a potato tuber). Hence one would expect that the virus of cucumber mosaic would fail to be carried over to the next generation in the seed. But this is one of the diseases which is sometimes transmitted by seed. The explanation of this anomaly is perhaps to be found in the work of Longo (*15*), who has shown that since the cucurbit embryo is so completely isolated by impermeable layers, an adaptation of a curious nature has been developed, in which the pollen tube opens a communication between the neck of the nucellus and the embryo-sac. Possibly the same channel could serve to transmit the virus to the embryo.

INSECT TRANSMISSION OF MOSAIC FROM THE WILD TO THE CULTIVATED CUCUMBER

Mosaic wild cucumber plants often occur at considerable distances from fields of cultivated cucurbits, and these and other wild host plants would be of relatively minor importance were it not for the fact that insects serve as carriers of the disease. Experiments have demonstrated, however, that the insects which transmit the disease in the case of the cucumber (*8*) also transmit mosaic from Micrampelis to the cultivated cucurbits.

Transmission of Mosaic from the Wild to the Cultivated Cucumber by the Cucumber Aphis

In these experiments, a number of cucumber aphids, *Aphis gossypii* Glover, were colonized on mosaic and healthy wild cucumber plants. The aphids from mosaic plants were later transferred to healthy cucumber plants under cages, and the aphids from healthy Micrampelis plants were placed on healthy cucumbers in other cages as controls. As shown in Table III, infection occurred on all plants to which aphids from mosaic wild cucumber plants were transferred, while the controls remained healthy in each experiment.

Table III.—*Transmission of cucurbit mosaic from Micrampelis lobata to healthy cucumber plants by Aphis gossypii*

Date	Condition of plants used as source of aphids	Number of plants	Number of mosaic plants	Date observed
July 14, 1919	Mosaic	4	4	July 23, 1919
Do	Healthy (control)	3	0	Do.
Aug. 18, 1919	Mosaic	7	7	Aug. 30, 1919
Do	Healthy (control)	3	0	Do.
July 10, 1920	Mosaic	8	8	July 21, 1920
Do	Healthy (control)	8	0	Do.
July 21, 1920	Mosaic	6	6	Aug. 2, 1920
Do	Healthy (control)	6	0	Do.
Aug. 9, 1920	Mosaic	6	6	Aug. 20, 1920
Do	Healthy (control)	6	0	Do.

Transmission of Mosaic from the Wild to the Cultivated Cucumber by the Striped Beetle and by the 12-Spotted Beetle

The transmission of mosaic from the wild to the cultivated cucumber by the striped beetle, *Diabrotica vittata*, and by the 12-spotted beetle, D. *12-punctata*, has been demonstrated by a number of experiments with both insects. The two species of Diabrotica are so similar in habit, however, that definite evidence of transmission by either species would indicate that both were concerned in carrying the disease.

In these experiments, beetles were collected at points supposedly distant from any sources of cucurbit mosaic infection, and then placed in cages containing healthy cucumber plants and allowed to feed for three days. The beetles were then removed and the plants were kept under observation for two weeks to determine whether any of the insects were carrying the mosaic virus when collected. In all cases, however, the test plants remained healthy. After the beetles had been tested in this manner, they were placed in cages containing healthy cucumber plants in 4-inch pots, together with a few potted plants of mosaic Micrampelis. The Micrampelis plants were so placed as not to be in contact with the cucumbers, thus insuring that no infection took place through contact of the leaves. The beetles were allowed to feed indiscriminately on both the cucumber and Micrampelis plants for periods ranging from three to seven days, and were then removed from the cages. Mosaic Micrampelis and healthy cucumber plants were placed in the control cages, but no insects were admitted. The results given in Tables IV and V indicate that the disease is readily transmissible by these insects under the conditions of these experiments.

TABLE IV.—*Transmission of cucurbit mosaic from Micrampelis lobata to healthy cucumber plants by Diabrotica vittata*

Date	Cage	Source of infection	Number of beetles in cage	Number of cucumber plants in cage	Number of mosaic plants	Date observed
May 6, 1919	1	1 mosaic Micrampelis plant in cage with healthy cucumbers.	25	6	3	May 15, 1919
Do	2	do	*0	6	0	Do.
June 14, 1919	1	3 mosaic Micrampelis plants in cage with healthy cucumbers.	30	5	2	June 30, 1919
Do	2	do	*0	5	0	Do.
June 11, 1920	1	do	20	8	3	June 30, 1920
Do	2	do	20	8	2	Do.
Do	3	do	20	8	1	Do.
Do	4	do	*0	10	0	Do.

* Control.

TABLE V.—*Transmission of cucurbit mosaic from Micrampelis lobata to healthy cucumber plants by Diabrotica 12-punctata*

Date	Cage	Source of infection	Number of beetles in cage	Number of cucumber plants in cage	Number of mosaic plants	Date observed
July 10, 1919	1	3 mosaic Micrampelis plants in cage with healthy cucumbers.	25	6	2	July 25 1919
Do	2	do	25	6	3	Do.
Do	3	do	*0	6	0	Do.
Aug. 15, 1920	1	1 mosaic Micrampelis plant in cage with healthy cucumbers.	30	6	3	Aug. 29, 1920
Do	2	do	25	4	1	Do.
Do	3	do	38	10	3	Do.
Do	4	do	*0	8	0	Do.

* Control.

Although the beetles used were first tested by allowing them to feed on healthy plants, there is a remote possibility that they may have carried infection from outside sources. This seems unlikely, however, as the experiments with *Diabrotica vittata* were conducted early in the spring at a time when no cultivated cucurbits had appeared in the vicinity, so that the only probable source of infection would have been mosaic plants on which the beetles had fed before they were collected. Other experiments, however, were in progress at the same time, in which beetles collected in the open were fed on healthy cucumber plants, but no infection occurred in such cases. The experiments with *Diabrotica 12-punctata* were necessarily conducted at a later date, as this species does not appear in any considerable numbers until after cucumbers in the field have passed the seedling stage. No mosaic was found on cucumbers, however, until some time after the insects had been collected, so that mosaic wild cucumber plants were, in all probability, the only source from which the insects may have carried infection. The preliminary tests with these insects, however, seem to have eliminated this possibility.

IMPORTANCE OF THE STRIPED CUCUMBER BEETLE IN THE TRANSMISSION OF MOSAIC FROM THE WILD CUCUMBER

The striped cucumber beetle is apparently of greater importance than either the 12-spotted beetle or the cucumber aphis in carrying

the disease from the wild cucumber to cultivated cucurbits. This is due to the fact that this insect emerges from hibernation each year at approximately the time at which the wild cucumber seedlings appear. Observations during the last five years have shown that the beetles may often be found feeding on the cotyledons of *Micrampelis lobata* when the first leaf is still only partially developed. The striped beetle occurs in considerable numbers in most cucumber-growing sections and is usually found on the wild cucumber throughout May and June. The beetles apparently feed on the wild cucumber and other host plants until the cultivated cucurbits come up, and the majority then appear to migrate to the cultivated hosts. This migration from the wild to the cultivated cucurbits probably continues to a small extent throughout the season. When wild cucumbers have been grown within a few yards of cultivated cucumbers, a constant flight from one host to the other has frequently been noted and, as will be shown later, it is probable that considerable distances may be covered between the wild and cultivated plants.

The lesser importance of the 12-spotted beetles in this relation is due to the fact that they are not as frequently found on Micrampelis in May and June, when compared with the numbers of *Diabrotica vittata*, as the 12-spotted beetle does not appear in any considerable numbers in the vicinity of Madison, Wis., until about July 1. On this account much of the experimental work has been done with *Diabrotica vittata*. There is no reason to believe that the 12-spotted beetle is not an agency in the dissemination of mosaic from the wild cucumber later in the season, and to some extent at an earlier date as well. In any case, the results with one species seem directly applicable to the other, if both species are present at the same time.

The cucumber aphis, *Aphis gossypii*, is probably not of much importance in carrying infection from the wild cucumber over any considerable distance. The aphids are rarely found on Micrampelis until after mosaic has appeared on cucumbers in the field and are not of common occurrence on the wild host, so that while they are almost certain to produce infection if they move from mosaic Micrampelis to the cucumber, their actual importance is limited. The winged generations are the only ones likely to cover any great distance and their migrations are apparently limited when compared to those of the striped beetle. In view of the greater numbers and activity of the beetles it is probable, therefore, that the aphids are of minor importance in the dissemination of the disease from the wild cucumber.

INFECTION OF CUCUMBER PLANTS BY STRIPED BEETLES COLLECTED ABOUT MOSAIC MICRAMPELIS PLANTS IN THE OPEN

The rôle of the striped beetle as a means of transmitting primary infection from the wild cucumber to the cultivated cucurbits has been demonstrated to some extent by experiments in which the beetles were collected from groups of Micrampelis plants containing mosaic individuals and placed in cages containing healthy cucumber plants. No effort was made to collect the beetles from mosaic plants alone and the insects were taken from all plants in the vicinity. The results of these experiments, as shown in Table VI, indicate that beetles from such mosaic centers were able to transmit the disease in a number of cases.

TABLE VI.—*Transmission of cucurbit mosaic to healthy cucumber plants by striped beetles collected from groups of mosaic Micrampelis plants*

Date	Number of beetles in cage	Number of plants in cage	Number of plants mosaic	Date observed	Date	Number of beetles in cage	Number of plants in cage	Number of plants mosaic	Date observed
June 15, 1919	74	16	3	July 1, 1919	May 28, 1920	30	12	0	June 14, 1920
Do	57	20	5	Do.	June 7, 1920	41	12	0	June 18, 1920
June 18, 1919	40	10	2	Do.	Do	52	15	2	Do.
Do	61	12	0	Do.	June 14, 1920	35	10	0	June 29, 1920
June 20, 1919	25	9	1	July 5, 1919	Do	35	10	0	Do.
June 29, 1919	40	12	0	July 12, 1919	Do	35	10	3	Do.
May 28, 1920	50	14	2	June 14, 1920					

The infection which occurred in 7 out of the 13 cages represents a fairly high percentage of infection in the case of the striped beetle, since many trials with beetles taken directly from mosaic cucumber plants have shown that relatively few such insects will transmit the disease. A considerable number of insects were placed in each cage, as the percentage of infection with the individual insects had always been low. This is in marked contrast to the results with the cucumber aphis, *Aphis gossypii;* but, as stated in an earlier paper (*8*), it is probably due partly to the fact that the beetle, a chewing insect, often attacks the stems and blossoms of the plant and, in a majority of cases, probably does not produce infection in wounds at these points. The results of artificial inoculation have shown that relatively little infection takes place through superficial injuries in the blossoms or on the surface of the stem.

DISTANCES OVER WHICH MOSAIC MAY BE TRANSMITTED FROM MOSAIC MICRAMPELIS PLANTS BY CUCUMBER BEETLES OR OTHER INSECTS

Although mosaic Micrampelis plants are a possible source of primary infection to cucurbits in the field, their importance in this connection rests to a great extent on the distances over which insects may transmit the disease from such plants. As already noted, the striped beetle, and to a lesser extent the 12-spotted beetle, seem the most important agents in such transmission, for they probably migrate more commonly and to greater distances than do aphids. An effort has been made to determine experimentally the distance which the disease may be transmitted by insects, in the belief that such transmission would most probably be through the agency of the cucumber beetle. Such experiments were necessarily only indicative, but were undertaken in the hope of accumulating some actual data on the spread of mosaic about a single group of mosaic wild cucumber plants.

An effort was made to remove all known or suspected host plants of cucurbit mosaic over a certain area, with the exception of a single group of mosaic Micrampelis plants which was left as a source of infection. Small plats of cucumbers were planted at varying distances from these wild cucumber plants, and under such conditions any mosaic occurring on the cucumbers could be traced with some certainty to the group of mosaic wild cucumbers. The area used for these experiments was nearly all under cultivation and almost free from known hosts of cucurbit mosaic. It extended north from

the mosaic Micrampelis for one-quarter mile to the shore of Lake Mendota, and one-half mile east and west. This area was carefully inspected to insure the removal of wild host plants, and it was believed any infection which occurred in the plats must be carried over a distance at least equal to that which separated them from the known source of mosaic.

These experiments were conducted on the same plan during 1920 and 1921. The number of plats was reduced in 1921, but those remaining occupied approximately the same positions as in 1920. Frequent inspections were made to note the first appearance of mosaic and as soon as it appeared in a plat all of the plants, both mosaic and healthy, were at once removed, except in one case which will be noted later. This was done to prevent the disease from spreading from plat to plat. The various dates of infection in the several plats are shown in Table VII.

TABLE VII.—*Results of experiments to determine distances over which mosaic infection may be carried by insects from Micrampelis lobata to cucumber*

1920				1921			
Plat	Distances from mosaic Micrampelis	Date beetles appeared in plat	Date mosaic appeared in plat	Plat	Distances from mosaic Micrampelis	Date beetles appeared in plat	Date mosaic appeared in plat
	Yds.				*Yds.*		
1a [a]	1	June 11	June 27	1a	1	June 8	June 21
2	140	...do.....	July 16	2b	175	...do.....	Aug. 28
3b	175	...do.....	Aug. 10	3c	225	...do.....	July 17
4c	225	...do.....	Aug. 2	4d	350	June 10	Aug. 18
5d	350	...do.....	July 23	5e	500	...do.....	June 30
6	350	...do.....	Aug. 2				
7e	500	...do.....	July 28				

[a] Letters indicate plats in approximately same location in 1920 and 1921; 1920 plats planted May 28 1920; 1921 plats planted May 28, 1921.

In both years many striped and a few 12-spotted beetles were already present when the seed was planted and the insects appeared in all the plats while the plants were still in the seedling stage. In both 1920 and 1921 the plants in plat 1, which was directly adjacent to the mosaic wild cucumbers, were infected at an earlier date than plants in any of the other plats. Observations showed that there were frequent migrations of beetles from the wild cucumbers to this plat. In both seasons there was an irregularity of infection as related to the distance from the supposed source of mosaic infection on Micrampelis. This is noticeable in plats 2 and 3 of the 1921 experiment, which were in an open field at a distance of approximately 50 yards from one another. Infection in plat 3 occurred on July 17, while in plat 2, which was nearer the wild cucumbers, the disease did not develop until August 28. The same fact is even more evident in the most distant plats of the two experiments, in both of which the disease developed much earlier on plants at a distance of 500 yards from the mosaic Micrampelis plants than in plats at half that distance. In the case of part 5, in the experiments of 1921, the infection recorded on June 30 consisted of a single plant, which was removed when first observed. As there was practically no possibility

of this infection being carried by those making the observations, and as no further infection appeared until late in August, it is quite probable that the original infection came from a distant source, such as the mosaic wild-cucumber plants, and that, owing to the chance nature of infection from beetles over such distances, no further infection occurred for some time.

Perhaps such an irregularity in infection is to be expected if we consider the beetles to have been the agency concerned in the transmission of the disease, for, as already stated, the percentage of infection due to these insects is always low as compared with that due to aphids. On this basis a certain element of chance may exist, and it is possible that a greater number of insects might visit a nearby plat without necessarily causing immediate infection, while a smaller number might reach a more distant plat and produce infection at an earlier date. In most cases it is improbable that great numbers of insects feed on Micrampelis and immediately travel to the cucumbers in the field. Recent investigations by Dudley, of the United States Bureau of Entomology (*12*) indicate that the beetles may travel as far as one-half mile and also that they perhaps pass over nearby cucumber fields when moving with the wind. Under such circumstances the above results probably are to be expected.

If we accept the results of these experiments at their face value, they indicate the possibility of infection being carried at least a quarter of a mile from groups of mosaic Micrampelis plants and at a date sufficiently early to allow for destructive development of the disease. In the plats within 350 yards of the Micrampelis plants, it was practically certain that infection must have been carried a distance equal to that of the known source on the wild cucumber. We can not be sure, however, that insects other than the cucumber beetles were not concerned in its transmission. This latter point is of minor significance, however, as regards the practical importance of the wild cucumber as a source of primary infection.

The source of infection for the most distant plats (plat 7 of 1920 and plat 5 of 1921), is less certain, perhaps, especially as the disease developed comparatively early at this point each year. These plats occupied approximately the same locations and were close to a small piece of wooded land which contained many wild plants. Careful inspection at various times failed to show any known host plants of cucurbit mosaic, but the possibility of their being overlooked is not beyond question. The general evidence, however, seems to indicate that infection was traceable to the wild cucumber. In theory, the distance over which the beetles may carry infection is limited only by the extreme range of their flight. Field observations, however, indicate that a range of 400 to 600 yards represents the limits within which there is a reasonable expectation of infection on the cultivated hosts, since at these distances fields have often remained free from mosaic for the greater part of the summer.

IMPORTANCE OF THE WILD CUCUMBER AS A SOURCE OF INFECTION OF THE CULTIVATED CUCURBITS

The present evidence has shown that the mosaic disease is carried over winter in the seed of the wild cucumber and that certain insects are able to transmit the disease from this host to the cultivated

cucurbits. The actual importance of the wild cucumber as a source of primary infection to the cultivated hosts is dependent, however, on the numbers of mosaic Micrampelis plants which occur in a given locality and their location with respect to fields of cultivated cucurbits. The factor of the distances over which insects carry the infection is, of course, equally important, but, for the present at least, it is assumed that this distance is approximately 400 to 500 yards. In most localities the striped beetle is always present in considerable numbers, so the presence of an insect carrier is assured.

Survey of 1919

If the wild cucumber is actually an important factor in overwintering mosaic, infected wild plants would be expected to occur in considerable numbers in districts where the mosaic disease is severe in the fields. Surveys of the past three years have shown that a distinct correlation of this sort exists in Wisconsin and others of the Central States, although the surveys have been chiefly confined to Wisconsin.

In these surveys all of the fields of cucumbers and other cucurbits in a given area were inspected for the presence of mosaic infection and an effort was made to locate as many groups of wild cucumber plants in the vicinity as possible. The occurrence of mosaic Micrampelis plants in different localities and the corresponding occurrence of mosaic on other cucurbits is shown in Table VIII. Aside from a few localities, however, no attempt was made to locate all of the mosaic wild cucumber plants in a given area. While the presence of the disease was noted, no attempt was made to show the number of cucumber fields visited or the number of these fields which were mosaic, because in sections visited late in the season there was naturally a greater amount of mosaic present than in localities visited when the disease had first appeared, as it had had an opportunity to spread from field to field during the intervening period. Under these circumstances the wild cucumber as a source of infection bears only slight relation to the extent of mosaic in the field, because only the early infection can safely be attributed to this source. The average number of fields visited in all localities was approximately 12, and in localities where the disease occurred mosaic was present in about 40 per cent of the fields.

The results of these surveys emphasized the fact that the wild cucumber occurs abundantly throughout the Central States. In many localities large groups of these plants occur along streams and in other moist situations and are found in smaller numbers in low places along roads, railway embankments, fence hedgerows, and other similar spots. The distribution of the plant has been greatly increased by the fact that it is often used as an ornamental, so that large numbers of wild cucumber plants are found about dwellings in most towns and villages. It is frequently found in neglected spots about farm buildings as a result of having been introduced as an ornamental, and a thorough survey in some localities has revealed many more plants than would be noted by a cursory inspection.

TABLE VIII.—*Occurrence of mosaic on Micrampelis lobata in various localities as related to the presence of the disease on cucumbers in the vicinity*

Date	Locality	Square miles of area covered	Number of centers of Micrampelis	Number of mosaic centers	Occurrence of mosaic on cucumbers in area
	Survey of 1919				
July 9	Sturgeon Bay, Wis.	1.5	7	0	0
July 10	Ellison Bay, Wis.	1.0	2	0	0
Do	Green Bay, Wis.	2.5	11	0	0
July 25	do	1.0	6	0	0
July 24	Menominee, Mich.	2.0	13	0	0
Do	Marinette, Wis.	1.5	11	0	0
July 11	Racine, Wis.	4.0	19	4	(a)
July 18	Madison, Wis.	5.0	24	10	(b)
July 22	Milwaukee, Wis.	1.5	6	0	(a)
Aug. 30	do	2.0	8	2	(a)
July 26	Portage, Wis.	3.0	8	3	(a)
Aug. 28	Ripon, Wis.	2.0	27	11	(b)
Aug. 29	Princeton, Wis.	2.0	30	19	(b)
Do	Fond du Lac, Wis.	0.5	5	1	(a)
	Survey of 1920				
June 11	Sparta, Wis.	1.0	8	5	(b)
July 24	Harvard, Ill.	2.0	18	8	(b)
July 26	Marengo, Ill.	1.5	11	5	(a)
Aug. 24	Huntington, Long Island	(a)	1	1	(a)
Aug. 25	Riverhead, Long Island	2.0	3	1	(a)
Aug. 26	Southampton, Long Island	(a)	1	1	(a)

[a] Indicates presence of mosaic. [b] Unusually severe mosaic infection. [a] No definite area covered.

As shown in Table VIII, no mosaic was found on cucumbers in the district around and north of Green Bay, in northwestern Wisconsin. There is no record of the disease in this section, although cucumbers are grown on a commercial scale in most of the localities visited. No mosaic was found on any wild cucumber plants in this part of the State. On the other hand, a survey over much of the cucumber-growing territory in the southern portion of Wisconsin revealed the more or less frequent occurrence of mosaic on the wild cucumber correlated with its presence on the cultivated cucurbits in the vicinity.

In many localities the disease was found on a considerable number of Micrampelis plants, while in other places it occurred on but few. As closely as could be estimated from observations of this type, there was a very striking correlation between the number of groups of mosaic plants of the wild cucumber and the extent of the disease in the fields, although, as previously stated, it was impossible to draw conclusions too definitely from observations made in various localities over a period of only six to seven weeks. It will be seen from Table VIII, however, that the disease was found on a number of wild cucumber plants at Madison, Ripon, and Princeton, Wis., during 1919, and that the disease was unusually severe in the fields at these points. Ripon and Princeton are of particular interest from the fact that the disease is known to have occurred in these localities as early as 1900, the earliest record of its appearance in Wisconsin. It has always been particularly severe in both places. The survey

of 1919 brought out the fact there were hundreds of mosaic wild cucumber plants in both towns and along the streams in the neighborhood. The number of mosaic centers and the total number of such plants found at both Ripon and Princeton was far in excess of that at most other points visited.

As considerable data were available on the previous losses from mosaic at most points visited, it was possible by this means to determine to some extent the probable relation of Micrampelis to infection in the field. In localities such as Ripon, Princeton, Madison, and Sparta in Wisconsin, and Harvard and Marengo in Illinois, where mosaic has long been a serious problem, there were considerable numbers of mosaic wild cucumber plants. In most of the other sections where there were fewer mosaic wild cucumbers, the disease has apparently been severe only in certain seasons, although some infection occurs each year.

The probable importance of the wild cucumber in overwintering mosaic was also emphasized by the fact long noted that the mosaic disease is more severe on cultivated cucumbers in the immediate vicinity of towns and villages than in outlying districts. This is probably due in part to the proximity of the fields to one another and the consequent ease of dissemination of the disease and in part to the fact that mosaic wild cucumbers are much more common about towns than in the country by reason of the greater use of the Micrampelis vine as an ornamental in and near the towns. It was also evident that mosaic on the cucumber was much more common in districts where there were numerous small streams and low places, which are the natural habitat of *Micrampelis lobata*.

Reports from other sections indicate that the wild host is common in all of the Central and Eastern States. Where surveys have been made in those States in sections in which the disease occurs, the presence of mosaic wild cucumbers has usually been established. The writers therefore consider that they are justified in regarding *Micrampelis lobata* as an important source of mosaic infection in most regions where cucurbits are grown on a commercial scale.

Recent investigations have shown that the milkweed (*Asclepias syriaca*) and the pokeweed (*Phytolacca decandra*) are also concerned in the overwintering of cucurbit mosaic and that they are frequently a source of primary infection to the cucurbits. Because of these other agencies by which the disease is carried over winter, the writers have not been able to definitely determine the comparative importance of a single host in this group, because their actual importance varies with the locality. It is evident, nevertheless, that the wild cucumber plays an important part in overwintering cucurbit mosaic.

MILKWEED AS AN AGENCY IN OVERWINTERING CUCURBIT MOSAIC

The existence of mosaic diseases on certain wild plants has been known for some time, but until 1920 there was no evidence that such diseases could be transmitted to the cucurbits. Jagger (*14*) had successfully transmitted cucumber mosaic to single species of the Compositae and Lobeliaceae, and one of the writers of this paper (Doolittle) had also brought about infection on *Martynia louisiana* Mill. (*8*), but aside from indicating that the disease was transmissible

to plants of other families than the Cucurbitaceae these results had little bearing on the problem of overwintering. More recent investigations, however, have shown that a number of plants of families outside the cucurbits are susceptible to cucurbit mosaic, and among these the milkweed, *Asclepias syriaca*, is an important agency in the overwintering of the disease.

FIELD OBSERVATIONS

The occurrence of mosaic on the milkweed was noted in 1916 by E. A. Bessey, of the Michigan Agricultural Experiment Station, who sent specimen plants to the senior writer. Inoculations were made to cucumber plants with this material, but no infection resulted. Milkweed plants which appeared to be mosaic were also found during 1917 and 1919, and a number of inoculations were made to the cucumber; but, prior to 1920 (*8*), there was no evidence, as far as the writers are aware, that milkweed mosaic was transmissible to the cucurbits. The studies with the wild cucumber, however, led indirectly to a further interest in the problem of the relation of the milkweed to the overwintering of mosaic.

At Madison, Wis.

During the summer of 1919, an effort was made to locate the source of primary infection in a field known as the Olin plat at Madison, Wis., which had been used for the experiments on the overwintering of mosaic in the soil. This plat was isolated and at a considerable distance from other cucumber fields, but it was noted that mosaic appeared there earlier and in more severe form than at any other point in the vicinity.

Several careful inspections failed to show the presence of mosaic wild cucumber plants within a mile of this point, although in most other mosaic-diseased fields about Madison it was possible to find wild cucumber plants sufficiently near to account for the infection. The early appearance of the disease on the Olin plat added to the peculiar aspect of the problem, and consequently all wild plants in the vicinity were examined for evidences of mosaic. It was found that the plat itself contained approximately 20 milkweed plants which showed marked symptoms of a disease similar to cucurbit mosaic. A further investigation of milkweeds in the neighborhood failed to show any other mosaic plants, and this fact added to the importance of the discovery, for it indicated a possible relation between the diseases on the two hosts. Such a possibility was of particular importance, as the milkweed is perennial and could therefore carry the disease over winter if susceptible.

The Olin plat was again planted to cucumbers in 1920, and record was made of the mosaic milkweed plants which appeared there. Approximately 20 such plants were found between the rows of cucumbers up to July 12, in addition to a considerable number of healthy plants. The first infection on the cucumber was noted July 12, and the field was thereafter inspected at intervals of three to four days. As the disease developed, record was made of the location of each mosaic plant and the proximity of mosaic milkweeds. These observations showed that practically all of the early infection on the cucumber had occurred within 6 to 10 feet of mosaic milkweeds, as shown by the accompanying field plan (fig. 1).

Aside from these plants, there was practically no mosaic infection on the cucumbers in this plat until about July 25. The cucumber aphis (*Aphis gossypii*) was present in considerable numbers from July 1 to the end of the season, and as a result 75 per cent of the cucumber plants became mosaic diseased between August 1 and August 15. The aphids were present on the mosaic milkweeds before the disease developed on the cucumber plants, and their presence on the milkweed supported the theory that this plant might be a source of primary infection to the cucumber, as the aphids were known to be carriers of the disease. If this were the case, the cucumbers directly adjacent to the mosaic milkweeds would probably be the first infected, for aphids ordinarily do not travel very far. As a result of these observations, it seemed probable that the milkweed might be of considerable importance in relation to cucurbit mosaic.

At Rockland, Wis.

Soon after the above observations were made, further evidence was obtained at Rockland, Wis., which eventually established the milkweed as a source of mosaic infection for the cultivated cucurbits. During 1920, experiments in regard to the possible control of cucurbit mosaic through the removal of wild cucumber plants were conducted at Rockland and all mosaic Micrampelis plants which could be found in the vicinity were removed during June and early July. Inspections were made about every two weeks and no mosaic was found on cucumbers in the vicinity until August 5. On this date one of the fields in the area from which wild cucumbers had supposedly been removed was found to be 90 per cent mosaic diseased. As only two other fields in the vicinity showed any trace of mosaic and as the infection was comparatively slight in both cases, the source of infection in the badly diseased field furnished a peculiar problem. A brief investigation, however, showed that nearly 100 mosaic milkweed plants were growing between the rows of cucumbers and that both the cucumbers and milkweeds were heavily infested with the cucumber aphis. Further inspection revealed the presence of mosaic milkweed plants in another of the less severely infected fields, and in this case also aphids were found on both hosts. The evidence at Rockland and Madison was so striking that cross-inoculation experiments were immediately begun in a further attempt to determine the possible identity of the mosaic diseases occurring on the two hosts.

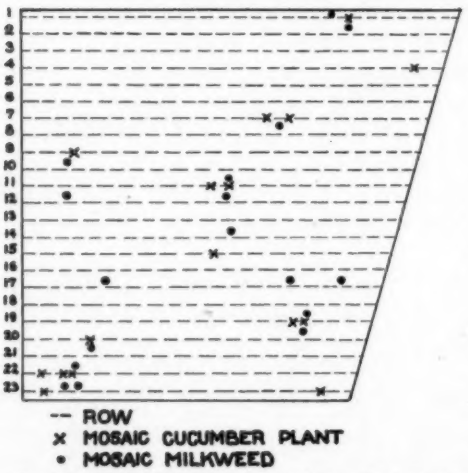


FIG. 1.—Plan of experimental plat at Madison, Wis., showing occurrence of first mosaic infection on cucumber plants directly adjacent to mosaic milkweeds. July 12 to 19, 1920

CROSS-INOCULATIONS FROM MOSAIC MILKWEED TO THE CUCUMBER

Inoculation with Aphids

Aphids from mosaic milkweeds found in the fields at Rockland were brought to Madison and transferred to healthy cucumber plants under cages in the greenhouse. Definite cases of mosaic occurred on all these plants, and the controls on which aphids from healthy milkweeds had been placed remained free from mosaic. These results were followed by a series of experiments in which aphids from healthy cucumbers were transferred to mosaic milkweed plants which were free from insects. These plants were covered with cheesecloth cages, and after the aphids had been allowed to feed on them for some time a number of the insects were transferred to healthy cucumber plants under cages in the greenhouse. This method eliminated the possibility that mosaic infection produced by aphids from mosaic milkweeds in the field might be due to aphids which had recently migrated from mosaic cucumber plants to the milkweed. The results given in Table IX show that nearly all cucumber plants inoculated in this manner developed typical cases of mosaic.

TABLE IX.—*Results of cross-inoculation from mosaic milkweeds (Asclepias syriaca) to healthy cucumber plants by means of Aphis gossypii*

Date, 1920	Source of inoculum	Method of inoculation	Number of plants inoculated	Number of mosaic plants	Date observed, 1920
Aug. 5	Rockland, Wis.	Aphids from mosaic milkweed	9	9	Aug. 12
Do	do	Aphids from healthy milkweed (control)	9	0	Aug. 18
Aug. 15	Madison, Wis.	Aphids from mosaic milkweed [a]	7	6	Aug. 27
Do	do	Aphids from healthy milkweed (control)	7	0	Sept. 1
Aug. 16	do	Aphids from mosaic milkweed [a]	6	5	Aug. 27
Do	do	Aphids from healthy milkweed (control)	6	0	Sept. 1
Sept. 1	do	Aphids from mosaic milkweed [a]	8	8	Sept. 19
Do	do	Aphids from healthy milkweed (control)	8	0	Do.
Sept. 4	do	Aphids from mosaic milkweed [a]	4	3	Sept. 13
Do	do	Aphids from healthy milkweed (control)	4	0	Do.
Sept. 5	do	Aphids from mosaic milkweed [a]	9	9	Sept. 20
Do	do	Aphids from healthy milkweed (control)	5	0	Sept. 23

[a] Aphids from healthy cucumber plants transferred to mosaic milkweed plants under cages in the field.

Artificial Inoculation

In addition to the cross-inoculation by means of *Aphis gossypii*, the susceptibility of the cucumber to the mosaic disease on the milkweed was confirmed by artificial cross inoculations. The crushed leaf tissues of mosaic milkweeds were used as inocula and a small fragment of the crushed leaf was inserted in a slight longitudinal cut in the lower portion of the stem of healthy cucumber plants. The oldest leaf of the inoculated plant was cut off close to the stem and the incision made in this wounded surface. The controls were treated in the same manner, except that the crushed leaf tissue of healthy milkweed plants was inserted in the stem. Inoculations were made both in the field and in the greenhouse and all plants inoculated were kept under cheesecloth cages to avoid outside infection. The results of these experiments, shown in Table X, have been uniformly successful, and the fact of the transmission of the mosaic disease from the milkweed to the cucumber is thus established.

TABLE X.—*Results of artificial cross inoculation of healthy cucumber plants with crushed leaf tissue of mosaic milkweeds*

Date (1920)	Source of inoculum	Location of inoculated plants	Number of plants inoculated	Number of mosaic plants	Date observed, 1920
Aug. 6	Rockland, Wis.	Greenhouse	8	3	Aug. 12
Do	do	do.[a]	8	0	Aug. 18
Do	do	Field	12	8	Aug. 12
Do	do	do.[a]	7	0	Aug. 18
Aug. 12	Madison, Wis.	Greenhouse	7	7	Sept. 1
Do	do	do.[a]	5	0	Do.
Aug. 13	do	Field	7	4	Do.
Do	do	do.[a]	9	0	Do.
Aug. 16	do	Greenhouse	6	4	Do.
Do	do	do.[a]	4	0	Do.
Do	do	Field	8	6	Do.
Do	do	do.[a]	7	0	Do.
Sept. 1	do	Greenhouse	8	5	Sept. 19
Do	do	do.[a]	8	0	Do.

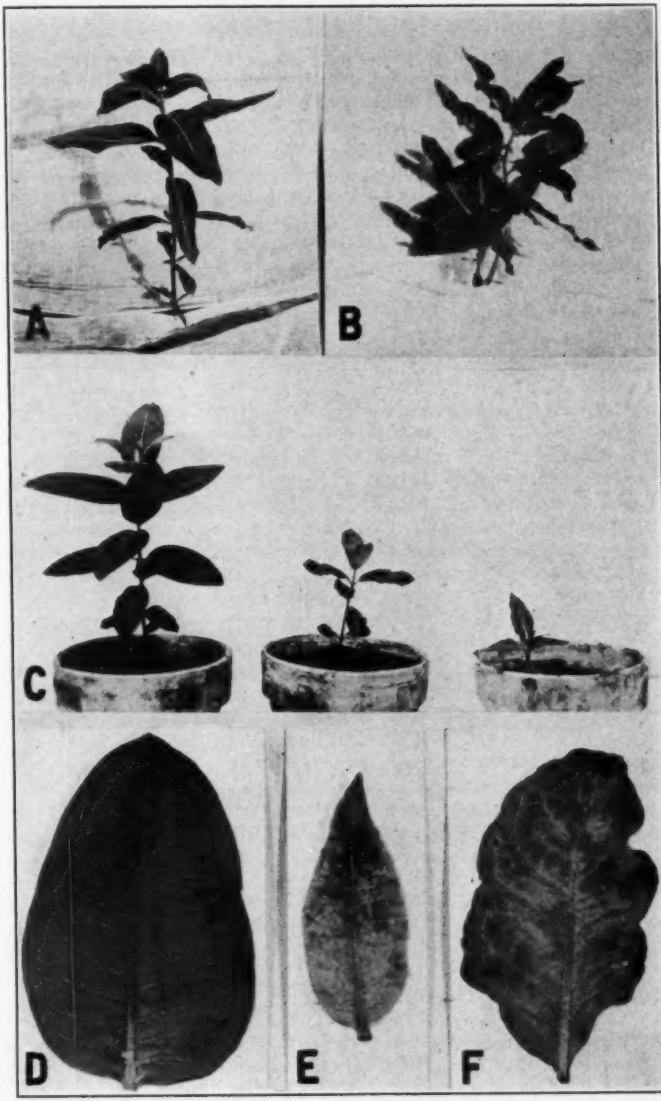
[a] Control.

CROSS INOCULATIONS FROM MOSAIC CUCUMBER PLANTS TO THE MILKWEED

The identity of the mosaic disease of the milkweed with that occurring on the cucurbits has also been shown by the results of inoculations from mosaic cucumber plants to healthy milkweeds. These inoculations have been relatively limited in number, but the results have been so definite as to amply demonstrate the possibility of such infection.

During the summer of 1920 cages were placed over four healthy milkweed plants, and aphids from a mosaic cucumber plant were colonized on each of them. Aphids from healthy cucumber plants were placed on four other healthy milkweeds as controls. These inoculations were made on August 12, and on September 1 the inoculated plants were found to show a curling and mottling of the leaves similar to the symptoms of mosaic milkweeds in the field. The controls, however, also showed a less severe curling of the leaves, although there was no change in color. In order to make sure that the apparent mosaic symptoms were not merely aphis injury, the aphids were removed from both the inoculated and control plants on September 4. The plants were then allowed to remain until September 29, when they were again examined. On this date the new growth of the inoculated plants showed evident symptoms of mosaic, while the later growth of the control plants was normal in character. The presence of mosaic was proved in the case of the inoculated plants by the transference of aphids from these plants to healthy cucumbers. Between September 2 and September 4, aphids from all the inoculated and control plants were transferred to healthy cucumbers under separate cages in the greenhouse. As shown in Table XI, the aphids from all of the inoculated milkweeds produced mosaic on the cucumbers, while the plants to which the aphids from control milkweeds were transferred remained normal. The success of these inoculations was further demonstrated by experiments herein described, in which the roots of these inoculated plants were grown in the greenhouse (pl. 2, B, C).

Further successful cross inoculations from mosaic cucumbers to the milkweed were made in experiments in the greenhouse during 1920–21. Five healthy milkweeds, grown from roots brought in



MOSAIC MILKWEED LEAVES AND PLANTS

A. Mosaic milkweed plant, *Asclepias syriaca*, found in cucumber plats at Madison, Wis., showing slight mottling and deformation of the leaves. Inoculations from this plant produced mosaic on healthy cucumber plants. Madison, Wis., August, 1922

B. Mosaic milkweed plant inoculated by means of aphids from a mosaic cucumber plant, showing extreme type of mosaic symptoms. Madison, Wis., August, 1920

C. Mosaic milkweed plants (at right) grown in greenhouse from roots of mosaic plant shown in B. Healthy plant (at left) grown from roots of healthy plant collected in the field. Madison, Wis., February, 1920.

D. Young leaf of healthy milkweed plant

E, F. Young leaves of mosaic milkweed collected at Rockland, Wis , July, 1922

from the field, were placed in cages and inoculated by means of aphids taken from mosaic cucumber plants. Healthy cucumber aphids were also placed on three other healthy milkweed plants as controls. The inoculations were made on February 4, and on February 24 all the aphids present on the plants were removed by spraying with nicotine sulphate. Aphids from healthy cucumber plants were transferred to both the inoculated and control milkweeds on March 1, and on March 7 some of the insects from each set of milkweeds were transferred to healthy cucumber plants under separate cages. The results of these inoculations, given in Table XI, show that infection occurred on the cucumbers on which aphids from the inoculated milkweeds were placed, while the aphids from the controls produced no infection.

TABLE XI.—*Results of cross inoculations of healthy cucumber plants from milkweed plants inoculated with cucurbit mosaic by means of aphids*

MILKWEED PLANTS INOCULATED IN FIELD, AUG. 13, 1920

Date of inoculation	Milkweed used as inoculum	Method of inoculation	Number of cucumber plants inoculated	Number of mosaic plants	Date observed
Sept. 1, 1920	1-1	Aphids	4	4	Sept. 13, 1920
Do	*23-1	do	4	0	Do.
Do	1-1	Crushed leaf tissue	4	3	Do.
Do	*23-1	do	4	0	Do.
Sept. 4, 1920	1-1	do	11	7	Sept. 15, 1920
Do	*1-4	do	10	0	Do.
Do	7-1	Aphids	4	3	Do.
Do	*1-4	do	4	0	Do.
Sept. 10, 1920	17-1	do	4	4	Sept. 19, 1920
Do	*23-2	do	4	0	Do.
Do	17-1	do	4	2	Do.
Do	*23-2	do	4	0	Do.
Sept. 12, 1920	17-1	do	5	5	Do.
Do	*22-1	do	5	0	Do.
Do	17-2	do	5	5	Do.
Do	*22-2	do	5	0	Do.

MILKWEED PLANTS INOCULATED IN GREENHOUSE, MAR. 20, 1921

Apr. 11, 1921	A-1	Crushed leaf tissue	8	3	Apr. 25, 1921
Do	A-3	do	8	4	Do.
Do	A-4	do	8	5	Do.
Do	A-5	do	8	0	Do.
Do	A-6	do	8	3	Do.
Do	*A-8	do	8	0	Do.
Do	*A-9	do	8	0	Do.

* Control plant.

The results of direct inoculation from the cucumber to the milkweed have been less uniformly successful, as only 2 definite cases of mosaic have been produced out of 15 plants inoculated in the field and greenhouse. These inoculations were made by inserting the crushed leaf tissue of a mosaic cucumber plant into longitudinal incisions at various points in the stems of healthy milkweeds and also by pricking the juices of mosaic cucumber plants into the younger leaves. The excessive flow of latex which follows the slightest wounding of the milkweed may account in part for the difficulty experienced in infecting by this method.

SYMPTOMS OF MOSAIC ON MILKWEED

The symptoms of the mosaic disease on the milkweed are similar in character to those found on the cucurbits. Mosaic plants are easily recognized by their dwarfed growth and mottled and distorted leaves (pl. 2, A, B). Mosaic milkweeds rarely reach a height of more than 2 feet, while normal plants usually attain 3 to 4 feet. Mosaic leaves are ordinarily mottled with irregular patches of greenish yellow. These areas are larger proportionately than in the case of the cucumber, and the mottled appearance is ordinarily more marked in character. This mottled character varies, however, with the individual plants and is occasionally much less pronounced (pl. 2, D, E, F).

The leaves of mosaic milkweeds are distorted in shape to an extent which is uncommon in the cucurbits, and the symptoms in this regard are more suggestive of the mosaic disease on tobacco. The leaves are ordinarily smaller than those of the normal milkweed and usually are more lanceolate than the oblong to ovate leaves of healthy plants. In some instances the leaves are of abnormal length and taper rapidly from a broad base to an almost filiform tip. Very commonly half of the leaf blade develops to almost normal size while the other half is only a few millimeters wide for all or part of its length. This effect of the disease produces abnormalities which are readily recognized. The leaves of mosaic plants also show a tendency to curl upward at the margins, producing a cuplike effect in extreme cases (pl. 2, B).

The dwarfed character of the plants, many of which are not more than 12 inches high, accompanied by the characteristic symptoms on the leaves, make the mosaic disease easily recognizable in the field. It has been found, however, that normal milkweed plants frequently show a mottling of the leaves which may be confused with the true mosaic. Such plants are of normal height, however, and show none of the curling or distortion characteristic of mosaic. The mottling in such cases is also different from that produced by mosaic in that it usually consists of areas which are limited by the parallel lateral veins and extends from the midrib to the edge of the leaf. These areas produce a striped effect, while in the case of mosaic, the yellowish areas are less regular in outline and are scattered irregularly over the leaf. Symptoms similar to those described above have also been observed on a single plant of *Asclepias amplexicaulis* Sm., but no infection was secured from inoculations made from this plant to the cucumber.

OVERWINTERING OF THE MOSAIC DISEASE IN THE MILKWEED

EXPERIMENTS WITH THE ROOTS OF MOSAIC MILKWEED

Since mosaic shoots are produced each year from the roots of other mosaic perennials such as the pokeweed (*Phytolacca decandra*) (*2*), it was expected that the mosaic disease would be carried from year to year in the roots of the milkweed. Observations in the Olin plat at Madison, Wis., during 1919 and 1920 showed that mosaic milkweeds appeared in the summer of 1920 at approximately the same points in which they had been noted during the preceding year. Experimental proof of such overwintering was obtained during the winter of 1920–21 in the case of roots of milkweeds brought in from the field and grown in the greenhouse. A number of mosaic and

healthy milkweed plants in the Olin plat were marked during the early fall, and in November a portion of the roots of these plants was dug and placed in 10-inch pots in the greenhouse. In most cases the roots of mosaic and healthy plants were grown in separate pots, but in two instances mosaic and healthy roots were planted together.

The roots in practically every instance sent up from one to two shoots during the early part of February and it was possible to determine the presence of mosaic on such shoots. From the results given in Table XII, which includes all such plants, it will be seen that the roots of mosaic plants produced shoots which were mosaic diseased in every case (pl. 2, C). The roots of the supposedly healthy plants, however, developed healthy shoots in all instances. In the two pots in which both healthy and mosaic roots were placed, both healthy and mosaic shoots were produced.

TABLE XII.—*Results obtained from growing roots of healthy and mosaic milkweed plants in the greenhouse at Madison, Wis., and inoculating healthy cucumbers from the plants thus produced*

Roots (brought in on Nov. 10, 1920)	Character of roots	Manner of infection	Type of shoots produced	Number of cucumber plants inoculated from shoots	Method of inoculation	Number of plants mosaic
1-1	Mosaic	Inoculated	Mosaic	4	Aphids	3
7-1	do	do	do	4	do	2
8-1	do	Natural	do	10	do	8
8-2	do	do	do		None	
9-1	do	do	do		do	
9-2	do	do	do	3	Aphids	1
12-1	do	do	do	2	do	0
12-2	do	do	do	3	do	2
13-1	do	Inoculated	do	9	Aphids and artificial.	8
13-2	do	do	do	7	do	3
14-2	do	Natural	do		None	
17-1	do	Inoculated	do	4	Artificial	3
17-2	do	do	do		None	
19-1	do	Natural	do	2	Aphids	1
19-2	do	do	do	2	do	2
9-H2[a]	Healthy		Healthy	4	do	0
10-1[a]	do		do	4	do	0
10-2[a]	do		do	6	Artificial	0
11-1[a]	do		do		None	
15-2	do		do		do	
15-3	do		do	4	Aphids	0
A-1	do		do		None	
A-3	do		do		do	
A-4	do		do		do	
A-5	do		do		do	
A-6	do		do		do	
A-7	do		do	8	Aphids	0
A-8	do		do	8	do	0
A-9	do		do	8	do	0
A-10	do		do		None	

[a] Roots of plants used as controls for plants indicated as "Inoculated" in Table XI.

These results, as shown in Table XII, were confirmed by a considerable number of inoculations from both the healthy and mosaic milkweeds to healthy cucumber plants. Inoculations in this case were made successfully both by means of aphids and by artificial inoculation with the crushed leaf tissues of the milkweed. It was also found that the roots of milkweeds inoculated with cucurbit mosaic in the field produced mosaic shoots in every case, while the roots of the controls of these experiments produced only healthy shoots.

These experiments demonstrate that milkweed plants when once infected with mosaic will produce mosaic shoots during succeeding seasons. Field observations during 1921 supported this belief, as mosaic milkweed plants were found during July, 1921, at all points where they occurred in 1920. These observations included the Olin plat at Madison, Wis., three fields at Rockland, Wis., and two at Marengo, Ill. In all of these fields the mosaic milkweed plants were found in the same spots during both 1920 and 1921, while at Madison the record also included 1919. In addition to these data, 12 mosaic milkweed plants were marked in the plat at Madison during the fall of 1920. Observations during June and July, 1921, showed that shoots of mosaic milkweed plants had appeared within 6 feet of nine of the points marked. In the other three instances no plants were seen during the season.

Trials with Seed from Mosaic Milkweeds

As already mentioned, mosaic milkweeds rarely produce seed. In a few cases, however, seed has been obtained from them, and experiments have been conducted to determine whether the mosaic disease is carried over winter in the seed as well as in the roots of mosaic plants. The seed from mosaic plants was grown in flats of sterilized soil in a greenhouse in which the temperature was held at approximately 28° C. Seed from healthy plants was grown in other flats in the same house as a control. Up to the present, 387 seedlings have been grown from the seed of mosaic milkweed plants, but no evidence of mosaic has been noted on any of these plants, many of which were grown until they had reached a considerable size. These results, therefore, indicate that the disease is not carried in the seed of mosaic milkweed plants.

Importance of the Milkweed as a Source of Cucurbit Infection

In view of the evidence just given, it is apparent that the milkweed is susceptible to cucurbit mosaic, and that the perennial rootstalks of mosaic milkweed plants carry the disease over the winter. As with the wild cucumber, however, the importance of the milkweed as a source of infection rests on the nature of the carriers which transmit the disease to the cultivated cucurbits, and upon the time of appearance, number, and location of the mosaic milkweeds.

Insect Carriers of Milkweed Mosaic

Aphids

The cucumber aphis (*Aphis gossypii*), as shown in this paper, is known to be an agent in transmitting mosaic from the milkweed to the cucumber. These aphids are commonly found on milkweed plants in the vicinity of cucumber fields infested with the insects, but are comparatively rare on milkweeds at a distance from cultivated cucurbits. Other species of aphids are often found on the milkweed, but up to the present no other species has been found which will feed on the cucumber. It seems probable, therefore, that in most cases the cucumber aphids reach the milkweed from infested cucurbits in the vicinity.

DIABROTICA BEETLES

Preliminary experiments with the striped and 12-spotted cucumber beetles, *Diabrotica vittata* and *D. 12-punctata*, indicate that these insects do not feed as readily on the milkweed as do the cucumber aphids. Both species of beetles, however, are found on the milkweed to a slight extent during the early summer, when cucurbits are not available as food. When caged with milkweed plants, both species attacked the plants to a certain extent, although the 12-spotted beetles feed more readily on the milkweed than the striped species. When striped beetles have been placed in cages containing mosaic milkweed and healthy cucumber plants, the cucumber plants in a few cases have become mosaic diseased, indicating that the beetle is capable of transmitting mosaic from the milkweed to the cucumber. The evidence to date is rather too meager to justify definite conclusions, but it seems probable that the beetles may occasionally carry infection from the milkweed to the cucumber. It is doubtful, however, whether they are of much importance as compared with the aphis as carriers of the disease from this host. No insects other than those mentioned above have been studied in relation to the transmission of milkweed mosaic.

OCCURRENCE OF MOSAIC MILKWEEDS IN THE FIELD

As the cucumber aphis seems to be the chief agency by which mosaic is transmitted from the milkweed to the cucurbits, it is probable that the mosaic milkweeds which occur in the immediate neighborhood of the fields are the only ones of importance as sources of primary infection. This belief is further strengthened by the fact that most cucumber aphids found on the milkweed have originally come from adjacent cucumbers and that these insects are comparatively limited in their flight.

Field observations during 1921 showed that mosaic milkweeds are frequently found in close proximity to fields of cucumbers, and also that such plants are rarely found in more distant locations. The field studies have been made principally at Madison and Rockland, Wis., and at Marengo, Ill. The surveys in these localities were confined to areas of from 1 to 3 square miles in which there were a number of cucumber fields. All the milkweeds which could be found in these areas during June and July were examined for evidences of mosaic. Later surveys were also made in the neighborhood of cucumber fields which developed mosaic.

At Madison only four groups of mosaic milkweed plants were found. The largest of these, already mentioned as occurring in the Olin plat, consisted of 27 plants, all of which were between rows of cucumbers. Approximately 100 milkweeds within 50 yards of the plat were examined, but no mosaic plants were found outside the cucumber plat. Four mosaic milkweed plants were found between the rows of cucumbers in another plat about a mile from the Olin field, and in this case also all milkweeds outside of the plat seemed free from the disease. Two other small groups of milkweeds showing mosaic were found in gardens in the neighborhood, both of which contained mosaic cucumber plants. Approximately 300 other milkweeds examined along roadsides and in open fields in the vicinity were all found to be healthy, so that up to the present the only mosaic milkweeds found at Madison have been in fields planted to cucurbits for several seasons.

At Rockland the fact that mosaic milkweeds were found near cucumber fields only was emphasized by surveys during 1921 and 1922. Over an area of 2 square miles, which included 10 cucumber fields, an effort was made to locate as many milkweed plants as possible and some hundreds of plants were examined during both seasons. Only two groups of mosaic plants were found at a distance of more than a few yards from cucumber fields. On the other hand, seven groups of mosaic milkweeds were found growing either in and immediately adjacent to fields of cucumbers or on land which had grown cucumbers in 1920. Two of these groups were at the same points where mosaic plants were found in 1920. The mosaic milkweeds at the different points varied in number from 3 to 100, and most of them were found between June 10 and July 20. In three instances the mosaic milkweeds were between rows of cucumbers. In four of the other six groups they were on land where mosaic cucumbers had grown in 1920. In all cases the mosaic plants were within 50 yards of cucumber fields, and as aphids were present during the latter part of July there was ample opportunity for the transmission of the disease to the cucumbers. It was not possible to trace the first mosaic infection on the cucumber to the milkweed as directly as in 1920, but in one field the first mosaic cucumber plants were found growing about a mosaic milkweed plant which had appeared late in the season.

At Marengo only five groups of mosaic milkweed plants were found during the season of 1921. The area covered was much larger than at Rockland, and the cucumber fields were scattered and at considerable distances from one another. Four of the five groups of mosaic milkweed plants were found on land which had grown cucumbers in 1920. Mosaic milkweeds were found in this field in 1920 and infection also occurred on the cucumbers during that year. In two of the four cucumber fields in which mosaic milkweeds were found in 1921, infection appeared three weeks earlier than in any of the remaining nine fields in the area examined. In every case where mosaic milkweeds were found the land had grown several successive crops of cucurbits.

At both Rockland and Marengo mosaic milkweed plants were found before cultivated cucurbits had appeared, so that this source of infection was present from the beginning of the season. Most of the mosaic milkweeds appeared during June and early July, but in some cases mosaic plants were found just breaking ground during the latter part of August. Aside from obtaining the data just referred to, little survey work has been done with regard to milkweed mosaic. Mosaic milkweeds were found in 1920 in three cucumber fields on Long Island, N. Y., in which nearly all of the cucumber plants were affected with mosaic. A further inspection of fields of other crops showed a large number of milkweeds in the vicinity, but no mosaic milkweeds were found at such points. Mosaic milkweed plants were also noted during 1921 at Sparta and Portage, Wis., and at Harvard, Ill.

It is evident from these observations that mosaic milkweeds, while comparatively rare, occur in most cases in the immediate vicinity of cucumber fields, or on land which has previously grown cucurbits. In view of the fact that practically no mosaic is found on milkweed plants in isolated situations or on farms where cucurbits are not

grown, it seems probable that the milkweed in most cases is first infected from the cucumber. This infection probably occurs chiefly through the agency of aphids and the mosaic persists in the milkweeds from year to year and thus furnishes a source of primary infection to nearby cucurbits.

COMPARATIVE IMPORTANCE OF THE MILKWEED AND WILD CUCUMBER IN OVERWINTERING CUCURBIT MOSAIC

When the milkweed was first discovered to be a factor in overwintering cucurbit mosaic, it was thought that it was probably of minor importance in comparison with the wild cucumber. Further investigations, however, have indicated that mosaic milkweeds are a serious menace to the cucurbits, and, as far as opportunity for infection is concerned, they are often of more importance than the wild cucumber. This belief is based on field observations made in the same localities for three seasons and is further substantiated by the results of experimental studies.

Mosaic wild cucumbers in the majority of cases occur at distances of from 100 to 500 yards or more from cucumber fields, except in the vicinity of towns, where they occasionally are very near the fields and are very numerous. In the case of this host, the Diabrotica beetles appear to be the chief means of disseminating the disease, as aphids are not commonly found on Micrampelis plants and do not cover as great distances as the beetles. It has also been found that only a relatively small percentage of the beetles which feed on mosaic wild cucumber plants are likely to transmit the disease to cultivated cucurbits. The field experiments previously described have also shown that at distances of 100 to 500 yards the infection of cucurbits from mosaic Micrampelis plants is of irregular occurrence. The whole question of infection from the wild cucumber depends on the factor of the transmission of the mosaic disease by the Diabrotica beetles, and the probability of the transmission of the disease from this host is apparently less than in the case of the milkweed.

The number of mosaic milkweed plants appears to be small when compared to the number of wild cucumber plants which have been found in the same localities, but in most cases the milkweeds occur either within or near the cucumber fields. Aphids are probably the most important means of transmitting the disease from the milkweed to the cucumber, and these insects will readily travel the distances which usually separate the mosaic milkweed plants from the cucumbers. Moreover, the aphids are practically certain to produce infection if transferred to healthy plants, while the Diabrotica beetles appear to transmit the disease only in a certain percentage of cases after feeding on mosaic plants. Although the situation varies with the locality, it is probable that the few mosaic milkweeds which do occur near the cultivated cucurbits are of more potential importance than a comparatively larger number of mosaic Micrampelis plants which are scattered over a wide area.

OVERWINTERING OF CUCURBIT MOSAIC ON THE POKEWEED

The presence of a mosaic disease on another perennial, the pokeweed (*Phytolacca decandra*), has been known for some time, but until recently there was no evidence that it was transmissible to plants of other families. Woods (*19*), in 1902, reported the occurrence of a

mosaic disease on this host, and Clinton (*4*), in 1915, stated that he had been unable to transmit pokeweed mosaic to healthy tobacco plants. Allard (*1*), in 1914, also mentioned pokeweed mosaic and reported that it was not transmissible to tobacco. In a later paper (*2*), Allard showed that the disease was readily transmissible to healthy pokeweed plants, but stated that he was unable to transmit it to tobacco or pepper. One of the writers (Doolittle) (*8*) also reported that he had been unable to transmit pokeweed mosaic to the cucumber. All of the inoculation experiments mentioned above were made by artificial methods, using the expressed juices or crushed tissues of mosaic plants.

The possibility of a relationship between the mosaic disease of pokeweed and that occurring on the cucurbits was first indicated by observations made by W. W. Gilbert at Holland, Mich., in 1915, where mosaic pokeweeds were found in abundance near a field of mosaic cucumbers. The first successful cross inoculations from the pokeweed to the cucumber were obtained in the course of experiments with the cucumber aphis.[4] Aphids from mosaic cucumber plants had been colonized on certain plants of species supposedly insusceptible to cucurbit mosaic, in order to determine whether the infective principle of the disease was transmissible to the offspring of aphids which had fed on mosaic plants. Among the plants used were a few pokeweeds, and it was noted that the majority of these plants showed symptoms of a mosaic disease within two weeks after the aphids had been placed on them. As a result of this evidence, further cross-inoculation experiments were undertaken, in which aphids were used as a means of inoculation.

METHODS OF INOCULATION

As already indicated by the experiments with the milkweed, the use of aphids as a means of inoculation has been the most important factor in demonstrating the wide host range of the disease. In the earlier studies of cucurbit mosaic (*8*) it was found that inoculations in which aphids were transferred from mosaic to healthy cucumber plants resulted in nearly 100 per cent infection, but that a considerably lower percentage of infection was obtained when plants were artificially inoculated with the crushed tissues or expressed juices of mosaic plants. The advantages of insects as a means of inoculation became more apparent in the cross-inoculation experiments described in this paper.

In the case of the milkweed, pokeweed, and martynia, earlier cross inoculation by artificial methods (*8*) had given only negative results, and it was supposed that these plants were not susceptible to cucurbit mosaic. The use of aphids as a means of inoculation, however, demonstrated later that cucurbit mosaic could be transmitted to all of these hosts and to several other plants of widely separated families. While infection has since been produced on many of these hosts by artificial methods of inoculation, the first infection has always resulted from insect inoculations and the results have been much more consistent than those obtained by the artificial method.

[4] The studies of the mosaic disease of pokeweed were conducted by the junior writer of this paper and presented as a master's thesis at the University of Wisconsin.

When aphids were used as a means of inoculation they were colonized on healthy cucumber plants under cages in the greenhouse or field. Healthy aphids from this stock were later transferred to the plant to be inoculated. The insects were ordinarily transferred by means of a camel's hair brush, and the number placed on each plant varied from 10 to 60. Ordinarily 10 to 15 aphids are found sufficient to insure infection, but in the earlier experiments with the pokeweed it was found that only a small percentage of the aphids remained on the plants after their transfer and the number used was therefore increased.

INFECTIOUS NATURE OF POKEWEED MOSAIC

As already shown by Allard (*2*), pokeweed mosaic is readily transmitted to healthy plants of this species by artificial inoculation. In order to be certain that the plants supposedly infected by means of the cucumber aphis were actually mosaic diseased, a number of inoculations were made from these plants to healthy pokeweeds, both by means of insects and by artificial methods. The results given in Table XIII show that the disease is readily transmissible to the pokeweed by either method of inoculation. The incubation period of the disease of the pokeweed, as in the case of that of the cucumber, varies to some extent with the rapidity of growth of the infected plant. The average incubation period appears to be from 5 to 10 days, and is therefore approximately identical with that of cucurbit mosaic.

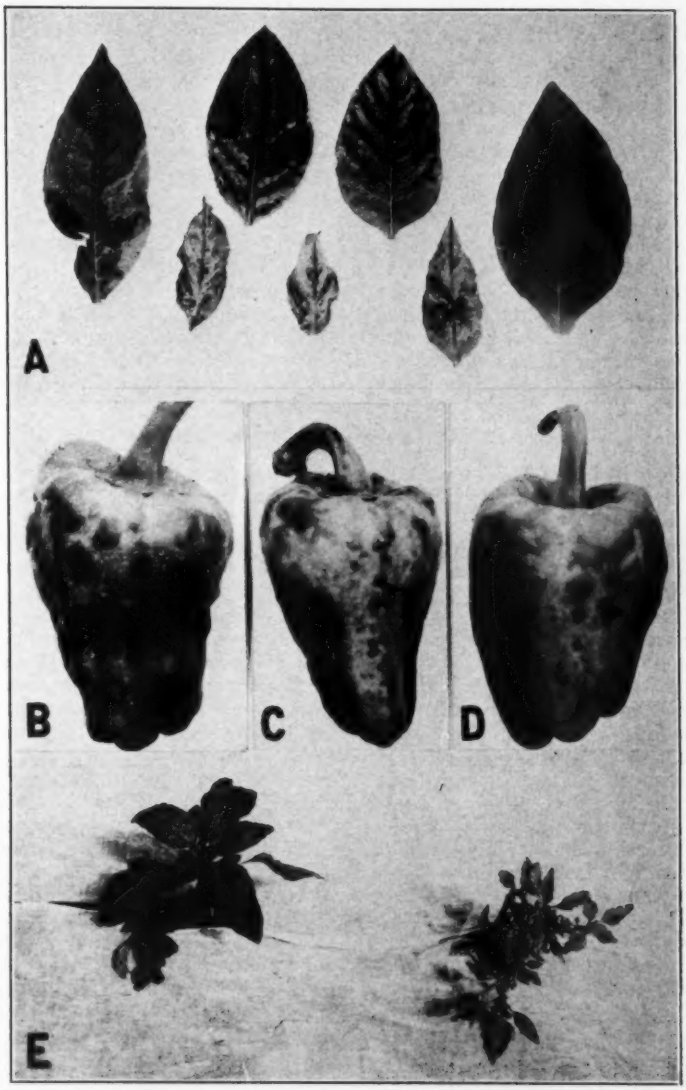
TABLE XIII.—*Results of inoculations of healthy pokeweeds from pokeweeds infected with cucurbit mosaic*

Date of inoculation	Source of inoculum	Number of plants inoculated	Number of plants mosaic	Date observed
	APHID INOCULATION *			
1922				1922
Mar. 15	Aphids from mosaic pokeweed	9	6	Mar. 30
Mar. 22	do	4	2	Apr. 7
Mar. 31	do	1	1	Apr. 10
	ARTIFICIAL INOCULATION			
Mar. 6	Crushed leaf tissue of mosaic pokeweed	6	2	Mar. 31
Mar. 22	do	8	8	Apr. 6
Do	Control	8	0	Do.
Mar. 23	Crushed leaf tissue of mosaic pokeweed	8	2	Apr. 11
Do	Control	8	0	Do.
Mar. 25	Crushed leaf tissue of mosaic pokeweed	3	1	Do.
Do	Control	3	0	Do.
Apr. 13	Crushed leaf tissue of mosaic pokeweed	24	20	Apr. 26
Do	Control	24	0	Do.
Apr. 24	Crushed leaf tissue of mosaic pokeweed	12	12	May 18
Do	Control	12	0	Do.

* Because of the fact that aphids had failed to colonize on the healthy plants intended for use as controls during these experiments, a number of healthy plants in the greenhouse were used as controls. None of the plants of this healthy stock developed mosaic.

SYMPTOMS OF MOSAIC ON THE POKEWEED

The symptoms of pokeweed mosaic are very similar to those of the mosaic diseases affecting the cucumber and tobacco. The disease first appears on the young leaves as a mottling of light greenish yellow in which the light areas are of small size. This mottling is accompanied by a downward curling of the midrib of the leaf, similar to



MOSAIC POKEWEED LEAVES, FRUITS OF MOSAIC PEPPER, AND MOSAIC PIGWEED PLANT

A. Young leaves of mosaic pokeweed infected with cucurbit mosaic. Healthy pokeweed leaf at right. Madison, Wis., 1922

B, C, D. Fruits of mosaic pepper plant, *Capsicum annuum*, showing dark green wartlike areas on surface. Madison, Wis., 1920

E. Mosaic plant of the pigweed, *Amaranthus retroflexus*, showing mottling of foliage and dwarfed growth. Inoculations from this plant produced mosaic symptoms on healthy cucumber. Healthy pigweed plant at left. Madison, Wis., August, 1922

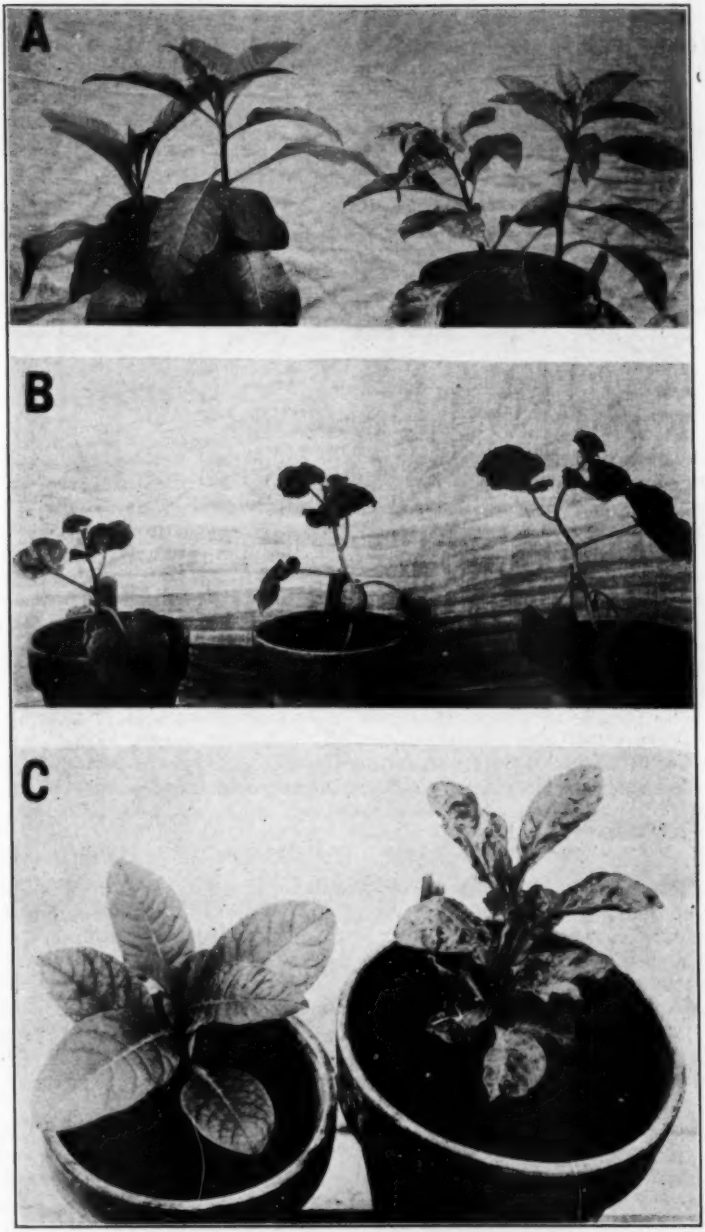
that found in mosaic cucumber plants. The light-green portions of the leaf may later include the greater portion of its surface. They are irregular in outline and do not appear to be delimited by the veins of the leaf (pl. 3, A). In the later stages of the disease the plants have a typically mosaic appearance, the darker portions of the leaf are slightly raised and the foliage has the wrinkled and blistered appearance which is associated with many mosaic diseases (pl. 4, A, C). The leaves of mosaic pokeweed plants are usually more irregular in outline than those of healthy plants. In the older plants the leaves are often curled upward at the edges in a manner similar to that of mosaic milkweeds.

Where plants are affected with the disease while young they become much stunted and blossom very sparingly. In the field, however, mosaic plants are commonly found which are 4 to 5 feet in height and appear to be making a vigorous growth. In all cases, however, the foliage shows the typical symptoms of the disease and the leaves are generally somewhat smaller than those of healthy plants.

SUSCEPTIBILITY OF THE POKEWEED TO CUCUMBER MOSAIC

As a result of the apparent infection of the pokeweed by aphids from mosaic cucumber plants, further cross inoculations were made from mosaic cucumber and muskmelon to the pokeweed, both by means of aphids and by artificial inoculation with the expressed juices of these plants. As shown by Table XIV, these experiments have shown that cucurbit mosaic is transmissible to the pokeweed by means of aphids from both the cucumber and muskmelon. Up to the present, however, no infection has been produced through wound inoculations with the expressed juices of these cucurbits. If only artificial methods of inoculation had been used the results of our earlier work, together with the results of Allard and Clinton in cross inoculation with tobacco, would have indicated that the pokeweed was not susceptible to other mosaic diseases and that the mosaic disease of pokeweed was distinct from that on the cucumber. It should be noted, however, that continued efforts at cross inoculation with cucurbit mosaic by artificial methods have eventually resulted in infection of host plants of other families, although earlier experiments had indicated that these hosts could not be infected except by means of aphids. It is possible, therefore, that the pokeweed may be infected with cucurbit mosaic by the use of artificial methods of inoculation. The use of the insect method is limited to some extent by the fact that it is not always possible to find insects that will transmit the disease in question and also feed on both the mosaic host plant and the one to be inoculated. The wide host range of the cucumber aphis, however, has made this insect of especial value in such experiments.

Some difficulty was encountered in the earlier experiments with the pokeweed, owing to the fact that during the winter the cucumber aphids often refused to feed on the pokeweed unless confined on the leaves in small cages. These cages were constructed by covering a Van Tieghem cell with fine wire screen and placing the cell over the leaf. The aphids were placed in the cage thus formed on the leaf and another piece of fine wire was placed on the under side of the leaf directly beneath the glass cell. The whole was then held in



MOSAIC POKEWEED AND MUSKMELON PLANTS

A. Mosaic pokeweed plants, *Phytolacca decandra* (at right) inoculated from a mosaic cucumber plant. Healthy control plants at left. Madison, Wis., April, 1923

B. Mosaic muskmelon plants (at left) inoculated by means of aphids from a mosaic pokeweed plant. Healthy control plant at right. Madison, Wis., 1923

C. Mosaic pokeweed plant (at right) inoculated with cucumber mosaic, showing extreme distortion and mottling of foliage. Healthy control plant at left. Madison, Wis., 1923

TABLE XIV.—*Results of cross inoculations from mosaic cucumber to healthy pokeweed plants by means of aphids*

Date of inoculation, 1922	Method of inoculation	Number of aphids per plant	Number of plants inoculated	Number of plants mosaic	Date observed, 1922
Mar. 15	Aphids from mosaic cucumber plant	16	3	0	Apr. 6
Apr. 6	do	15	6	5	Apr. 14
Do	Aphids from healthy cucumber plant (control)	15	2	0	Do.
Apr. 17	Aphids from mosaic cucumber plant	15	4	0	May 8
Do	Aphids from healthy cucumber plant (control)	15	2	0	Do.
May 8	Aphids from mosaic cucumber plant	25	8	5	May 17
Do	Aphids from healthy cucumber plant (control)	25	4	0	Do.
July 16	Aphids from mosaic cucumber plant	25	4	1	July 25
Do	Aphids from healthy cucumber plant (control)	25	2	0	Do.
July 29	Aphids from mosaic cucumber plant	30	12	8	Aug. 7
Aug. 3	do	30	12	6	Aug. 11
Do	do	(a)	3	2	Aug. 9
Do	Aphids from healthy cucumber plant (control)	25	12	0	Do.

[a] Aphids transferred naturally from mosaic cucumber plant in same cage.

place by a clamp, the screen on the under leaf surface preventing crushing by the clamp. This method proved successful, but after a time it was found that if a large number of aphids were colonized on the plants a few would remain without being confined in any way. It was also noted that the offspring of the aphids which remained on the pokeweed would feed more readily on this host than did the parent insects. The use of cages was therefore abandoned as unnecessary. It was later found that aphids would colonize readily on the pokeweed in large numbers during the spring and summer, possibly on account of some change in the juices of the pokeweed owing to its more vigorous and succulent growth.

The results of the inoculations by means of aphids were not as consistent with the pokeweed as with some other hosts, owing to the earlier difficulty in the colonization of the insects on the plants. The evidence, however, shows that the mosaic disease of cucurbits may be readily transmitted to the pokeweed.

TRANSMISSION OF MOSAIC FROM THE POKEWEED TO THE CUCURBITS

Reciprocal inoculations have also been made from mosaic pokeweed plants to the cucumber and muskmelon. In these inoculations the writers were again able to produce infection when aphids were used as a means of inoculation but were unsuccessful in producing infection by artificial inoculations. The results, as shown in Table XV, indicate that both the muskmelon and cucumber are susceptible to pokeweed mosaic (pl. 4, B). This being the case, it is evident that the pokeweed represents another perennial wild host of cucurbit mosaic.

OVERWINTERING OF CUCURBIT MOSAIC ON THE POKEWEED

TRIALS WITH ROOTS OF MOSAIC PLANTS

Since the pokeweed is a perennial and is known to be susceptible to cucurbit mosaic, it would be expected that the disease would live over winter in the roots of infected plants. Field observations in Michigan during 1915, 1916, and 1923, and at Anna, Ill., during 1922 and 1923, have shown that the roots of mosaic plants will again

TABLE XV.—*Results of cross inoculations from mosaic pokeweeds to healthy cucumber and muskmelon plants by means of aphids*

Date of inoculation, 1922	Method of inoculation	Number of aphids per plant	Number of plants inoculated	Number of plants mosaic	Date observed, 1922
	Cucumber				
Feb. 15	Aphids from mosaic pokeweed plant	25	1	1	Feb. 20
Feb. 24	....do	10	4	0	Mar. 13
Do	Aphids from healthy pokeweed plant (control)	10	2	0	Do.
Mar. 15	Aphids from mosaic pokeweed plant	15	2	0	Mar. 22
Do	Aphids from healthy pokeweed plant (control)	15	2	0	Do.
Mar. 16	Aphids from mosaic pokeweed plant	15	2	1	Mar. 20
Mar. 20	....do	15	10	9	Mar. 24
Do	Aphids from healthy pokeweed plant (control)	15	4	0	Do.
July 10	Aphids from mosaic pokeweed plant	15	8	4	July 16
Do	Aphids from healthy pokeweed plant (control)	15	4	0	Do
Aug. 21	Aphids from mosaic pokeweed plant	(a)	49	25	Aug. 31
Do	Aphids from healthy pokeweed plant (control)	(a)	28	0	Do.
Aug. 22	Aphids from mosaic pokeweed plant	15	12	11	Do.
Do	Aphids from healthy pokeweed plant (control)	15	12	0	Do.
Aug. 24	Aphids from mosaic pokeweed plant	15	15	11	Do.
Do	Aphids from healthy pokeweed plant (control)	15	12	0	Do.
	Muskmelon				
Aug. 4	Aphids from mosaic pokeweed plant	30	3	3	Aug. 14
Do	Aphids from healthy pokeweed plant (control)	30	3	0	Do.
Aug. 25	Aphids from mosaic pokeweed plant	15	12	9	Sept. 1
Do	Aphids from healthy pokeweed plant (control)	15	12	0	Do.

[a] Aphids allowed to transfer naturally from mosaic and healthy pokeweed plants in the respective cages.

produce mosaic shoots during the following year. Roots from such plants were collected at Anna in 1923 and taken to Madison, Wis. In all cases they produced mosaic shoots, and infection was produced by inoculation from these plants to the cucumber. Pokeweeds inoculated from mosaic cucumber plants were planted in the open at Madison in the summer of 1922 and the plants were allowed to overwinter. Most of these plants were winterkilled, but the few which survived produced mosaic shoots during the spring of 1923. It has also been found that when the top of a mosaic plant is cut back to the roots the new shoots are always mosaic diseased. It appears, therefore, that the mosaic disease which affects the pokeweed in the field is transmissible to the cucurbits and that the disease is carried over winter in the roots of the mosaic plants.

TRIALS WITH SEED FROM MOSAIC POKEWEEDS

In addition to the trials with roots of mosaic plants, seed from mosaic pokeweeds has also been tested in order to determine whether the disease is carried over winter in this manner. A considerable amount of seed was collected for the writers by E. A. Bierbaum at Anna, Ill., during the summer of 1922, from both mosaic and healthy pokeweeds. As in the case of the trials with seed from the milkweed, the seed was planted in flats of sterilized soil in the greenhouse and grown at a temperature of approximately 28° C. Seed from healthy pokeweed plants was used as a control. These trials included about 800 seedlings from mosaic plants, but there was no evidence that the disease is carried in the seed. This evidence of nontransmission of pokeweed mosaic in the seed is in keeping with observations made by W. W. Gilbert at Donaldson, Ind., in 1922, and by the senior author at Anna, Ill., in 1923. At both points it was found that cucumber

fields near mosaic pokeweed plants contained a number of pokeweed seedlings, all of which were free from mosaic. Practically all of the old pokeweeds in the vicinity, however, were severely affected with mosaic.

INSECTS AS AGENCIES IN THE DISSEMINATION OF POKEWEED MOSAIC FROM THE POKEWEED TO THE CUCURBITS

Aphids

It was first thought that the cucumber aphids would feed on the pokeweed only when forced to do so by a lack of other host plants. This belief was at first confirmed by an inspection of mosaic pokeweeds in southern Illinois during the summer of 1922. No aphids or other insects were found on the pokeweed plants examined at that time, although they showed signs of insect injury at some earlier date. No aphids were found in the surrounding cucumber fields, however, so the evidence in this case was not conclusive. There was some question as to whether there was any common insect carrier of the disease from the pokeweed to the cucumber, and unless such a carrier existed it was not certain that the pokeweed was an important source of infection to the cucurbits. Later in the summer, however, it was found that the cucumber aphids were present in considerable numbers on pokeweeds in the experimental plats at Madison, and that their occurrence was not due to artificial conditions. The aphids had appeared naturally on the cucumbers and later had apparently migrated to the near-by pokeweed plants. The natural transference of the aphids from one host to the other was also demonstrated by experiments in which five mosaic pokeweed plants were placed in separate cages which also contained a number of healthy cucumber plants. The experiments were conducted in the field, and care was taken to see that the pokeweed and cucumber plants were not in contact. In each of these cages the mosaic pokeweeds soon became covered with aphids from the cucumbers, and in every case the cucumber plants in the cage became mosaic diseased within 12 days after the pokeweeds were introduced. In the control cages in which healthy pokeweeds had been placed there was no mosaic infection on the cucumbers in any case.

The migration of aphids from cucumbers to the pokeweed was also demonstrated by experiments in which healthy pokeweed plants were placed in three cages containing mosaic cucumber plants which were infested with aphids. In these experiments all of the pokeweeds became covered with the aphids, although they were not in contact with the cucumbers, and later developed symptoms of mosaic. These observations and experiments indicate that the cucumber aphis is a carrier of mosaic from the pokeweed to the cucurbits and that the aphids may also be responsible for a portion of the mosaic infection found on the pokeweed. The situation in the case of pokeweed seems much like that of the milkweed, although the evidence of the transmission of mosaic from the pokeweed to the cucurbits in the field is not as complete as that which exists with regard to the milkweed, owing to the fact that it has been necessary to conduct most of the work in Wisconsin, where the pokeweed is of rare occurrence in the field.

Other Insects

The only other insects which have been tested as possible carriers of pokeweed mosaic are the striped and 12-spotted Diabrotica beetles. In experimental trials with these insects the writers have found no evidence which indicates that they are agents in the transmission of mosaic from the pokeweed, but the work so far has been of a limited nature. Both insects have occasionally been found feeding on the pokeweed and are often found between the young leaves at the tip of the plants, although there is little evidence to indicate any extensive feeding on this host. At present the available evidence does not indicate that they are important factors in transmitting the disease to the cucurbits.

IMPORTANCE OF THE POKEWEED AS A SOURCE OF INFECTION TO THE CUCURBITS

In Wisconsin and northern Illinois the pokeweed is of little importance in overwintering cucurbit mosaic because of its rare occurrence. Up to the present the writers have never found pokeweed plants in the vicinity of cucumber fields in Wisconsin. In southwestern Michigan, however, mosaic pokeweeds have been observed in the vicinity of cucumber fields in Allegan, Berrien, Ottawa, and Van Buren Counties. The disease has been noted in abundance by W. W. Gilbert in the vicinity of Donaldson, Ind., where cucumber mosaic is also common. M. W. Gardner, of the Indiana Agricultural Experiment Station, reports that mosaic pokeweeds are abundant in the vicinity of melon fields at Vincennes, Ind., where cucurbit mosaic has been serious for some time. In the summers of 1922 and 1923 one of the writers visited fields of cucumbers at Anna, in the extreme southern portion of Illinois, which had suffered severely from mosaic for several seasons. Pokeweed plants were found to be numerous along the roadsides and in fence rows about the fields. At least 50 per cent of the plants observed were affected by mosaic, and in many cases they occurred within a few feet of cultivated cucurbits. As the pokeweed appears to be a potential source of infection to the cucurbits, the observations in the Anna district indicate that the opportunities are far greater for infection from this host than in any of the localities where the milkweed and wild cucumber are apparently responsible for overwintering mosaic.

The occurrence of such extensive infection on the pokeweed was of special interest, as neither the wild cucumber nor the milkweed seemed to occur in the locality. A careful search revealed only one group of plants of *Asclepias syriaca*, and these were at a distance of some miles from cucumber plantings and were free from mosaic. No wild cucumber plants were found in the vicinity and the local growers seemed unfamiliar with both the wild cucumber and milkweed. Gardner also reports that he has seldom seen either the wild cucumber or milkweed in southern Indiana. As these hosts are not common in the vicinity of Anna, it seems probable that the mosaic infection on the cucurbits is traceable to the mosaic pokeweeds in the vicinity of the fields. Further observations have made this theory seem more probable, since mosaic pokeweeds have been found growing in hotbeds which contained young cucumber plants ready for setting out in the field. These pokeweeds were so

large that they must have been present for at least three seasons prior to the observations. Under these conditions, the presence of aphids would be very likely to result in infection of the young cucumber plants and the disease would in this way be introduced early in the season. The field evidence, therefore, indicates that the pokeweed should be classed with the wild cucumber and milkweed as a source of primary infection in some localities.

PRESENT STATUS OF THE PROBLEM OF OVERWINTERING

From the work described above, it is evident that the importance of the various wild host plants varies with the locality. Up to the present, however, all of our observations in Wisconsin, Indiana, and Illinois have shown that mosaic infection is common on at least one of the above wild host plants in all districts where the disease is prevalent on the cultivated cucurbits. The overwintering of mosaic on the wild cucumber, milkweed, and pokeweed seems to be established, but it is possible that the list of host plants may be increased by further investigations.

Recent experiments have confirmed the work of Muncie (*16*), which indicates that the catnip, *Nepeta cataria* L., may also be a perennial host of cucurbit mosaic. The importance of this host in the field in Wisconsin has not yet been determined. Studies by one of the authors (Walker) have also indicated that certain perennial species of Physalis are susceptible to cucurbit mosaic and the possibility of other sources of infection is indicated by the results of cross inoculations with annual plants of other families. These experiments have shown that cucurbit mosaic may be transmitted to a wide variety of host plants and have furnished evidence which indicates that certain supposedly distinct mosaic diseases may be identical. If this is the case, the possible sources of infection for the cucurbits might perhaps be considerably increased in number. The results of these experiments with annual host plants of the cucurbits and of other families are given in the following section of this paper.

CROSS-INOCULATION EXPERIMENTS WITH CUCURBIT MOSAIC

CROSS INOCULATIONS FROM THE CUCUMBER TO OTHER SPECIES OF THE CUCURBITACEAE

It has been shown in earlier papers (*8*, *11*) that many species of the Cucurbitaceae are susceptible to cucurbit mosaic. In these earlier experiments all of the cucurbits tested proved to be susceptible to the disease, with the exception of those in the genus Citrullus. In this genus, which includes the watermelon and citron, infection was produced on the green-seeded citron only. The results with this latter host were also confirmed by the work of Jagger (*14*). These cross-inoculation experiments have since been continued and a considerable number of additional species and varieties, including many European, Asiatic, and African cucurbits, have been found to be susceptible to the disease. The earlier results have been confirmed in that all varieties except the genus Citrullus have proved to be susceptible to the disease. Inoculation of many varieties of watermelon and citron, however, have never resulted in infection, except in the case of the green-seeded citron just mentioned.

The results of these experiments are given in Table XVI, together with a list of the named varieties known to be susceptible to cucurbit mosaic.

TABLE XVI.—*List of cucurbits inoculated with cucurbit mosaic*

Genus and species	Number of varieties inoculated	Number of varieties found susceptible	Source of seed
Cucumis sativus L. (cucumber)	44	44	United States (26), Europe (8), China (4), India (2), Africa (4).
Cucumis anguria L.	1	1	United States.
Cucumis grossulariaeformis Hort	1	1	United States.
Cucumis metuliferus Mey	2	2	United States (1), Africa (1).
Cucumis odoratissimus Moench	1	1	United States.
Cucumis ficifolia Bouche	2	2	India.
Cucumis melo L. (muskmelon)	17	17	United States (14), Persia, (1), Ceylon (1), Africa (1).
Cucumis melo var. dudaim Naudin	1	1	United States.
Cucumis melo var. utilissima Roxbg	1	1	India.
Cucumis melo var. flexuosus Naudin	1	1	United States.
Cucurbita moschata Duchesne (pumpkin)	2	2	United States.
Cucurbita pepo L. (pumpkin)	10	10	United States (5), Africa (5).
Cucurbita pepo var. condensa Bailey (squash)	6	6	United States (4), India (2).
Cucurbita pepo var. ovifera Bailey (gourd)	5	5	United States.
Cucurbita maxima Duchesne (gourd)	7	7	United States (4), India (3).
Lagenaria vulgaris Ser. (gourd)	5	5	United States.
Lagenaria leucantha Rusby	1	1	United States.
Luffa cylindrica (aegypta) Roehm	6	6	United States (4), India (2).
Luffa acutangulis Roxbg	2	2	United States (1), India (1).
Benincasa cerifera Cog	1	1	United States.
Benincasa hispida Cog	1	1	United States.
Bryonopsis laciniosa Naudin	1	1	United States.
Ecballium elaterium A. Rich	1	1	United States.
Micrampelis lobata (Michx.) Greene	1	1	United States.
Momordica charantia L.	5	5	United States (2), India (3).
Momordica involucrata E. Meyer	1	1	United States.
Sicyos angulatus L.	1	1	United States.
Trichosanthes anguina L.	5	5	United States (2), India (3).
Citrullus vulgaris Schrad. (watermelon and citron)	21	21	United States (8), China (2), Africa (11).

The following-named varieties of the more important cultivated cucurbits have been found susceptible to cucurbit mosaic. Only the varieties from the United States and Europe have been listed, as most of the Asiatic and African varieties tested were known only by species names.

CUCUMBER (*Cucumis sativus*): Chicago Pickling, Cumberland, Davis Perfect, Earliest of All, Early Cyclone, Early Fortune, Early Frame, Early Green Cluster, Early Russian, Fordhook Pickling, Giant Pera, Green Prolific, Heinz Pickling, Japanese Climbing, Jersey Pickling, Klondyke, Lemon, Livingston's Emerald, Long Green, Milwaukee Pickling, Parisian Pickling, Small Gherkin, Snow's Pickling, Telegraph, Thorburn's Everbearing, White Spine.

France: Cornichon de Meaux, Cornichon de Toulouse, Vert de Paris.

Holland: Half Long Prolific, King William, Oblong Green Pickling.

Germany: Short Green Early, Short Green Parisian.

CUCUMIS ANGURIA: West Indian gherkin.

MUSKMELON (*Cucumis melo*): Banana Citron, Banquet, Emerald Gem, Hackensack, Hearts of Gold, Honeydew, Long Yellow, Mango, Netted Gem, Orange Christiana, Osage, Rocky Ford, Shumway Giant, Winter Pineapple.

PUMPKIN (*Cucurbita moschata*): Large Cheese, Winter Crookneck.

PUMPKIN (*Cucurbita pepo*): Golden Oblong, Japanese Pie, Mammoth King, Small Sugar, Vegetable Marrow.

SQUASH (*Cucurbita pepo* var. *condensa*): Cocozell Bush, Delicata, Summer Crookneck, White Scallop Bush.

SQUASH (*Cucurbita maxima*): Mammoth Hubbard, Mammoth Whale, Striped Custard.

GOURDS (*Cucurbita pepo* var. *ovifera*): Dipper, Egg, Orange, Pear, Turk's Turban.
GOURDS (*Lagenaria vulgaris*): Bottle, Calabash, Hercules Club, Spoon, Sugar Trough.
GOURDS (*Luffa cylindrica*): Dish Cloth, Sponge.
CITRON (*Citrullus vulgaris*): Green-seeded citron.

The following varieties of watermelon and citron (*Citrullus vulgaris*) have been found to be apparently immune to cucurbit mosaic:

WATERMELON: Halbert Honey, Kleckley Sweet, Mammoth Santiago, Olds 1908, Tom Watson, Sweet Heart.
CITRON: Red-seeded citron.

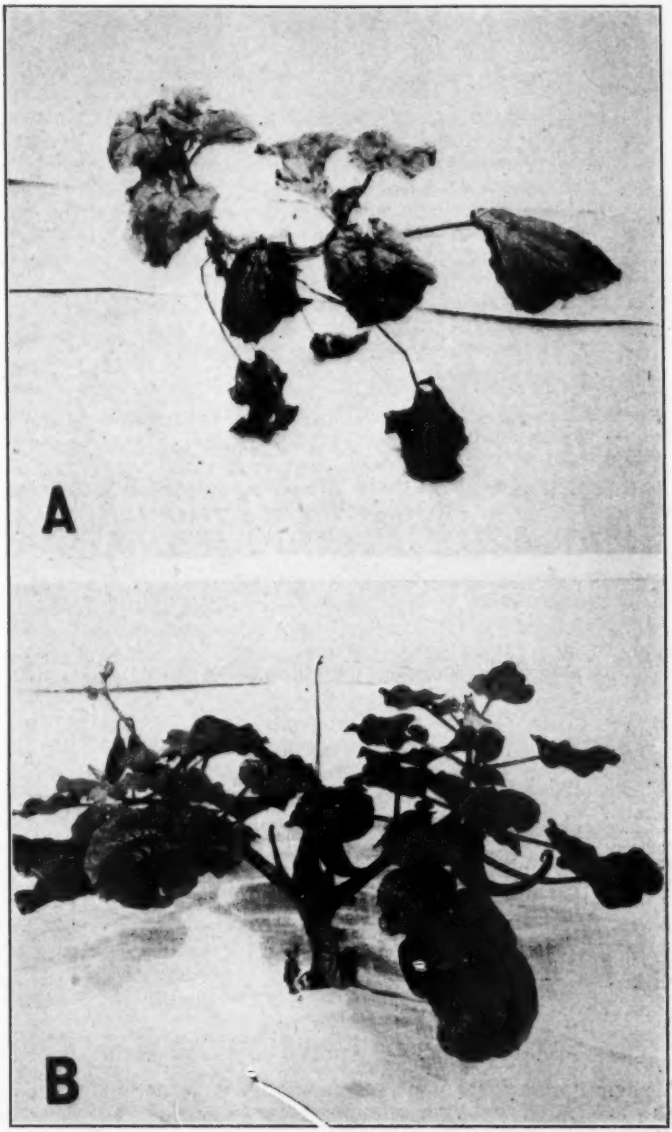
From 8 to 50 inoculations have been made with each variety, using both insects and artificial methods of inoculation. A considerable number of cross inoculations have also been made between the various species found to be susceptible to the disease. All but one variety of those tested in the genus Citrullus seemed to be nonsusceptible to the disease. As far as the writers are aware, no evidence of mosaic infection has ever been obtained on watermelons inoculated either by means of aphids or by artificial methods, although many varieties from many parts of the world have been used in these experiments. Since both Allard (*1*) and Nishimura (*17*) have presented evidence which tends to show that certain species of the Solanaceae may carry the infective principle of mosaic without showing visible symptoms, a number of inoculations were made to both cucumber and muskmelon from watermelons which had been inoculated with cucurbit mosaic. These inoculations were made both by artificial methods and by aphids, chiefly the latter, but no evidence was obtained which would indicate that the watermelon may carry the disease without the appearance of visible symptoms.

CROSS INOCULATIONS FROM THE CUCUMBER TO PLANTS OF OTHER FAMILIES

EXPERIMENTS WITH MARTYNIA

The susceptibility of the martynia (*Martynia louisiana*) to cucurbit mosaic has been briefly noted in an earlier paper (*8*). Although earlier inoculations indicated that the disease could not be transmitted to this host, it was later found that the martynia could be readily infected with mosaic when aphids were used as a means of inoculation (pl. 5, A, B). A continuation of these experiments has given definite evidence of the susceptibility of the martynia to cucurbit mosaic. Further experiments have also shown that the disease may be transmitted from the cucumber to the martynia by artificial inoculations as well as by aphids. The results of these inoculations are given in Table XVII.

The susceptibility of the martynia to cucurbit mosaic under field conditions has been demonstrated by experiments in which martynia plants were grown between rows of cucumbers in the field. The cucumbers developed mosaic early in the season, and a number of the martynias also became infected with the disease after its appearance on the cucumber. Inoculations from these plants to healthy cucumbers resulted in a high percentage of infection. The susceptibility of the cucumber to the mosaic disease found on the martynia has also been proved by inoculations from martynia plants inoculated from mosaic cucumbers as well as from those found naturally infected in the field. As shown in Table XVIII, the disease is transmissible from the martynia to the cucumber by either method of inoculation.



HEALTHY AND MOSAIC MARTYNIA PLANTS

A. Mosaic martynia plant, *Martynia louisiana*, inoculated by means of aphids from a mosaic cucumber plant

B. Healthy martynia plant used as control in above experiment. Madison, Wis., August, 1921

TABLE XVII.—*Results of inoculations of healthy plants of Martynia louisiana with cucurbit mosaic*

Date of inoculation	Method of inoculation	Location of plants	Number of plants inoculated	Number of plants mosaic	Date observed
Sept. 2, 1919	Aphids from mosaic martynia	Greenhouse	9	6	Sept. 12, 1919
Do	Aphids from healthy martynia (control).	do	8	0	Sept. 20, 1919
Apr. 10, 1920	Aphids from mosaic martynia	do	5	3	Apr. 30, 1920
Do	Aphids from healthy martynia (control).	do	5	0	Do.
Apr. 15, 1920	Artificial	do	6	4	Apr. 24, 1920
Do	Control	do	6	0	Do.
Apr. 28, 1920	Aphids from mosaic martynia	do	3	3	May 10, 1920
Do	Aphids from healthy martynia (control).	do	3	0	Do.
July 8, 1920	Artificial	Field	8	5	July 21, 1920
Do	Control	do	8	0	Do.
July 22, 1920	Aphids from mosaic martynia	do	6	2	Aug. 3, 1920
Do	Aphids from healthy martynia (control).	do	6	0	Do.
Aug. 8, 1920	Aphids from mosaic martynia	do	4	4	Aug. 24, 1920
Do	Aphids from healthy martynia (control).	do	4	0	Do.
Aug. 12, 1920	Artificial	Greenhouse	8	6	Do.
Do	Control	do	8	0	Do.

TABLE XVIII.—*Results of inoculation of healthy cucumber plants from mosaic plants of Martynia louisiana*

Date of noculation	Method of inoculation	Location of plants	Number of cucumber plants inoculated	Number of plants mosaic	Date observed
July 23, 1919	Aphids from mosaic martynia	Greenhouse	12	12	Aug. 4, 1919
Do	Aphids from healthy martynia (control).	do	12	0	Do.
Sept. 3, 1919	Artificial	do	8	6	Sept. 14, 1919
Do	Control	do	8	0	Do.
Do	Artificial	Field	10	8	Do.
Do	Control	do	8	0	Do.
July 22, 1920	Aphids from mosaic martynia	Greenhouse	8	5	July 31, 1920
Do	Aphids from healthy martynia (control).	do	8	0	Do.
July 29, 1920	Artificial	do	6	3	Aug. 7, 1920
Do	Control	do	6	0	Do.
Do	Artificial	Field	10	5	Do.
Do	Control	do	10	0	Do.
Aug. 2, 1920	Artificial	Greenhouse	6	5	Aug. 13, 1920
Do	Control	do	6	0	Do.
Aug. 10, 1920	Aphids from mosaic martynia	do	4	3	Aug. 18, 1920
Do	Aphids from healthy martynia (control).	do	4	0	Do.
Oct. 10, 1920	Artificial	do	6	4	Oct. 27, 1920
Do	Control	do	6	0	Do.
Aug. 10, 1922	Artificial	Field	5	3	Aug. 18, 1922
Do	Control	do	5	0	Do.
Aug. 11, 1922	Artificial	do	6	4	Aug. 22, 1922
Do	Control	do	4	0	Do.

SYMPTOMS OF CUCURBIT MOSAIC ON THE MARTYNIA

The symptoms of the disease on martynia are very similar to those on the cucurbits. Mosaic martynia plants develop a curling and mottling of the younger leaves which is much like that which occurs on the cucumber, with the exception that the green areas are very large and few in number, the yellowed portion including the greater part of the leaf. It has also been noted that a single branch of a mosaic martynia plant will show striking symptoms of mosaic, while

the remainder of the plant shows no evidence of the disease. Inoculations from various parts of such plants to healthy martynias have indicated that the infective principle is localized in the branches, which show visible symptoms of the disease. Eventually, however, the entire plant develops definite symptoms of mosaic. While these cases are somewhat abnormal in martynia, they are of great interest, since in all other plants which have been infected with cucurbit mosaic, the symptoms of the disease manifest themselves at all growing points at approximately the same time. Mosaic martynia plants are much dwarfed when infected with mosaic and the older leaves wilt and die in a manner very similar to those of the cucumber (pl. 5, A, B). In most cases the fruits are much dwarfed but show no mottling, but in one instance plants were found whose fruits were slightly mottled with yellow and showed wartlike protuberances very similar to those found on mosaic cucumber fruits.

Experiments with Pepper Plants

Cross inoculations from mosaic cucumbers to the pepper

The appearance of a mosaic disease on pepper plants (*Capsicum annuum L.*) which were growing in the vicinity of mosaic cucurbits led to the first cross inoculations with this host. The cucumber aphis will occasionally feed on the pepper, and it was found that cucurbit mosaic could be transmitted to the pepper by means of these insects. As in the case of martynia, the disease was later successfully transmitted to the pepper by artificial methods of inoculation, but the percentage of infection has always been considerably lower by this method than when aphids were used. The results of the inoculations from the cucumber to the pepper are given in Table XIX. As in the case of martynia, there is considerable field evidence of the transmission of mosaic from the cucumber to the pepper. Where pepper plants are grown next to mosaic cucumber plants in the field, a large percentage of the plants have always shown symptoms of mosaic before the end of the season. The experimental plat in which these experiments were conducted was at a considerable distance from any fields of tobacco or tomatoes and there was no reason to believe that the infection on the pepper had come from any source other than the cucumber.

Cross inoculations from mosaic peppers to the cucumber

Cross inoculations from mosaic peppers to healthy cucumber plants have been successful, as shown by the data in Table XX. These inoculations were made from peppers inoculated from mosaic cucumber plants and also from those found naturally infected with mosaic in the field. The inoculations from naturally infected pepper plants included those grown near mosaic cucurbits and presumably infected from that source and also mosaic plants found at other points where there were no mosaic cucurbits in the neighborhood. Infection was brought about both by artificial methods of inoculation and by the use of aphids, but the latter method was considerably more successful although artificial inoculations have given a considerably higher percentage of infection than in the case of the inoculations from cucumber to pepper.

TABLE XIX.—*Results of cross inoculation of healthy pepper plants with cucurbit mosaic*

Date of inoculation	Method of inoculation	Location of plants	Number of plants inoculated	Number of plants mosaic	Date observed
May 8, 1920	Aphids from mosaic cucumber plant	Greenhouse	6	4	May 15, 1920
Do	Aphids from healthy cucumber plant (control).	do	6	0	Do.
May 18, 1920	Aphids from mosaic cucumber plant	do	6	5	May 27, 1920
Do	Aphids from healthy cucumber plant (control).	do	6	0	Do.
May 28, 1920	Aphids from mosaic cucumber plant	do	8	8	June 10, 1920
Do	Aphids from healthy cucumber plant (control).	do	8	0	Do.
July 19, 1920	Aphids from mosaic cucumber plant	do	4	4	Aug. 2, 1920
Do	Aphids from healthy cucumber plant (control).	do	4	0	Do.
uly 20, 1920	Aphids from mosaic cucumber plant	do	7	7	July 28, 1920
Do	Aphids from healthy cucumber plant (control).	do	7	0	Do.
uly 23, 1920	Artificial	do	4	3	July 31, 1920
Do	Control	do	4	0	Do.
July 24, 1920	Artificial	do	6	2	Aug. 2, 1920
Do	Control	do	6	0	Do.
July 29, 1920	Artificial	Field	19	8	Aug. 10, 1920
Do	Control	do	9	0	Do.
Do	Artificial	Greenhouse	6	2	Do.
Do	Control	do	6	0	Do.
Aug. 2, 1920	Aphids from mosaic cucumber plant	do	6	6	Aug. 10, 1920
Do	Aphids from healthy cucumber plant (control).	do	6	0	Do.
Aug. 4, 1920	Aphids from mosaic cucumber plant	do	6	6	Aug. 11, 1920
Do	Aphids from healthy cucumber plant (control).	do	6	0	Do.
Aug. 18, 1920	Artificial	do	8	3	Sept. 1, 1920
Do	Control	do	8	0	Do.
Do	Aphids from mosaic cucumber plant	do	8	6	Do.
Do	Aphids from healthy cucumber plant (control).	do	8	0	Do.
Sept. 2, 1920	Artificial	Field	18	8	Sept. 23, 1920
Do	Control	do	18	0	Do.
Aug. 10, 1922	Artificial	do	12	5	Aug. 22, 1922
Do	Control	do	8	0	Do.
Aug. 14, 1922	Aphids from mosaic cucumber plant	do	8	8	Aug. 24, 1922
Do	Aphids from healthy cucumber plant (control).	do	8	0	Do.

TABLE XX.—*Results of cross inoculations from mosaic pepper to healthy cucumber plants*

Date of inoculation	Method of inoculation	Location	Number of plants inoculated	Number of plants mosaic	Date observed
Apr. 28, 1920	Aphids from mosaic pepper plant	Greenhouse	18	18	May 15, 1920
Do	Aphids from healthy pepper plant (control).	do	14	0	Do.
July 8, 1920	Aphids from mosaic pepper plant	Field	3	3	July 20, 1920
Do	Aphids from healthy pepper plant (control).	do	3	0	Do.
July 19, 1920	Aphids from mosaic pepper plant *	do	6	6	Aug. 1, 1920
Do	Aphids from healthy pepper plant (control).	do	6	0	Do.
Mar. 26, 1922	Aphids from mosaic pepper plant	Greenhouse	18	13	Apr. 7, 1922
Do	Aphids from healthy pepper plant (control).	do	10	0	Do.
May 1, 1922	Aphids from mosaic pepper plant	do	28	21	May 10, 1922
Do	Aphids from healthy pepper plant (control).	do	14	0	Do.
May 11, 1922	Artificial	do	8	4	May 25, 1922
Do	Control	do	8	0	Do.
June 10, 1922	Artificial	do	10	5	June 21, 1922
Do	Control	do	10	0	Do.
Aug. 8, 1922	Aphids from mosaic pepper plant *	do	4	4	Aug. 21, 1922
Do	Aphids from healthy pepper plant (control).	do	4	0	Do.
Apr. 15, 1923	Artificial	do	4	2	Apr. 25, 1923
Do	Control	do	4	0	Do.

* Pepper plants found naturally infected in the field.

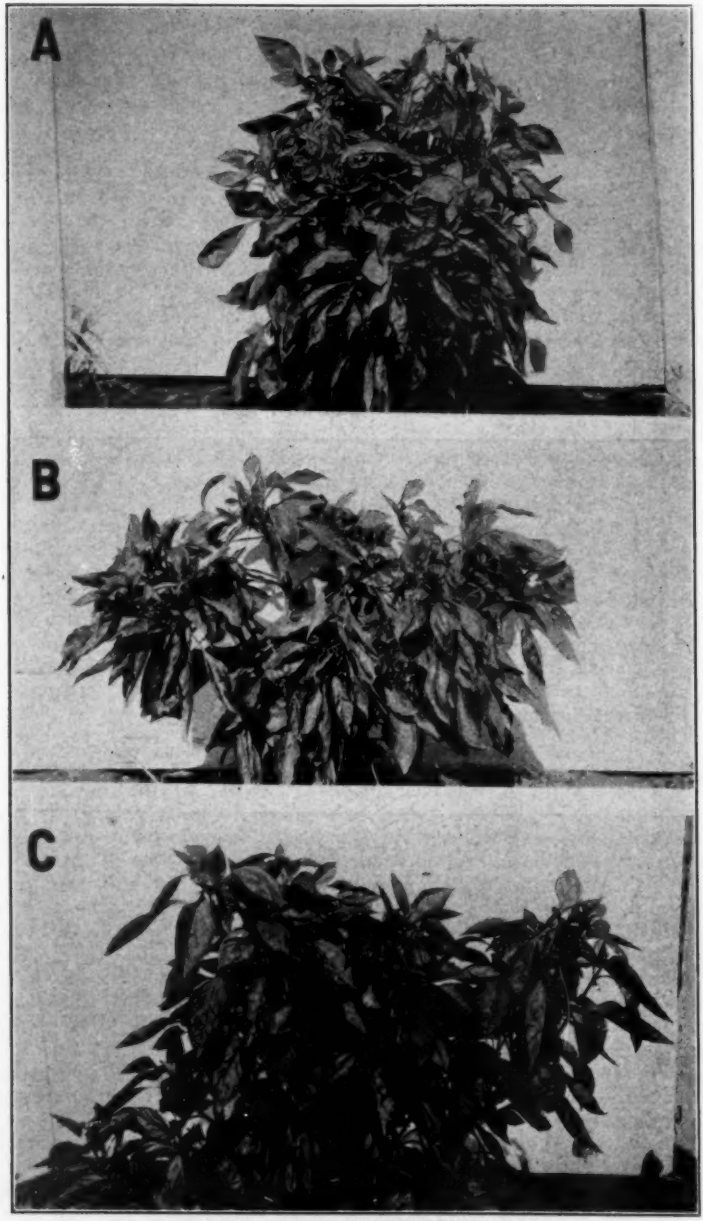
SYMPTOMS OF CUCURBIT MOSAIC ON THE PEPPER

When inoculated with cucurbit mosaic, the symptoms of the disease develop in the pepper in a manner which is very similar to that noted in the cucumber. The younger leaves of the infected plant curl downward along the midrib and the basal portion of the leaf is frequently a lighter green than that at the tip. Within a short time, however, such leaves develop the mottled appearance characteristic of the disease. The contrast in color, however, is seldom as pronounced as in the cucurbits and the mottling of the leaves is not general as in the cucumber, as only the very young leaves and those produced after infection are affected in this way. When pepper plants have been mosaic diseased for some time, the leaves are considerably smaller than those of healthy plants of the same age. The stem internodes are shortened considerably and the length of the petioles is somewhat reduced. Mosaic plants have a more compact habit of growth than normal plants, and the leaves are often abnormally narrow and drawn out in filiform fashion at the tip (pl. 6, A). This character of growth often produces an almost rosettelike character in plants which are infected when young. The foliage of mosaic peppers becomes a yellowish green, and the leaves appear to be firmer in texture than those of healthy plants. The mottling of the leaves is also less pronounced in plants which have been infected for some time and may be so slight as to be almost indistinguishable except upon close examination. In a few cases the fruits of mosaic pepper plants have shown symptoms which are comparable to those found on the fruits of the cucumber (pl. 3, B, C, D). The greater part of such fruits retain their normal green color, but the surface is broken with dark-green areas which are raised above the surface in warty swellings similar to those found on cucumbers affected with mosaic.

INTERTRANSMISSION OF CUCUMBER AND TOBACCO MOSAIC

The discovery of the susceptibility of the pepper to cucurbit mosaic has been of particular interest, since both Allard (*1*) and Schwarze (*18*) have shown that the pepper is also susceptible to the mosaic disease of tobacco. A comparative study was made, therefore, between pepper plants inoculated with tobacco mosaic and those inoculated from mosaic cucumber plants. These experiments indicated that pepper plants inoculated from mosaic plants of either of these hosts behaved in essentially the same manner. The period of incubation was the same and the symptoms appeared to be of the same type in both cases. The plants were dwarfed, and the leaf symptoms were the same as those described above (pl. 6, A, B, C). The pepper is also susceptible to tomato mosaic and it was found that peppers inoculated from mosaic tomato plants also showed symptoms apparently the same as those found on peppers inoculated from mosaic cucumber plants. This would be expected, in view of the results with tobacco, the infective agency of tobacco and tomato mosaic being apparently the same.

Since the pepper was susceptible to both cucumber and tobacco mosaic, the possibility was suggested that the mosaic of one of these hosts might be transmitted to plants of the other host through the



HEALTHY AND MOSAIC PEPPER PLANTS

A. Mosaic pepper plant inoculated with cucumber mosaic, showing distortion of foliage and slightly dwarfed character of growth

B. Mosaic pepper plant inoculated with tobacco mosaic on same date as plant in A, and showing similar symptoms

C. Healthy pepper plant used as control in above experiment. Madison, Wis., August, 1923

pepper. Such a possibility was of interest, owing to the fact that a large number of inoculations had been made from mosaic cucumber plants to tobacco, and vice versa, without obtaining any evidence that either of these mosaic diseases was transmissible to the other host. The symptoms on the pepper were so similar, however, when the plants were inoculated either with tobacco or cucumber mosaic, that it seemed possible that the diseases on tobacco and cucumber were at least intertransmissible if not identical.

Experiments were undertaken, therefore, in which pepper plants were inoculated with cucurbit mosaic, both by means of aphids and by artificial methods of inoculation. The plants used in these experiments were grown in a greenhouse which contained no other mosaic plants and were kept covered with cheesecloth cages during the experiment. Control plants were kept under separate cages in each series of inoculations. After mosaic symptoms had developed on the peppers thus inoculated with cucurbit mosaic, inoculations were made from them to healthy cucumber and tobacco plants. The same pepper plant was used as a source of inoculum for both hosts and control plants were placed under separate cages. In the case of the cucumber, the inoculations were made by means of aphids and also by artificial methods, using the juices of the mosaic pepper. The tobacco plants were inoculated only by the latter method. In all of these experiments, as shown in Table XXI, a high percentage of infection occurred on both cucumber and tobacco plants when inoculated from peppers affected with cucurbit mosaic. The controls remained healthy in all cases. The symptoms produced on the tobacco plants appeared to be the same as those produced on other tobacco plants inoculated with tobacco mosaic for comparison, and the incubation period was the same in both cases. This also held true in the case of the incubation period of cucumbers inoculated from mosaic cucumber plants when compared with those inoculated from the pepper.

TABLE XXI.—*Results of cross inoculations of cucumber and tobacco plants from pepper plants infected with cucumber mosaic*

Date of inoculation, 1922	Pepper plant used as inoculum	Plant inoculated	Method of inoculation	Number of plants inoculated	Number of plants mosaic	Date observed, 1922
Aug. 19	2-e (mosaic)	Tobacco	Artificial	10	6	Aug. 27
Do	1 (healthy)	do	Control	10	0	Do.
Do	2-e (mosaic)	Cucumber	Artificial	6	4	Aug. 26
Do	do	do	Aphids from mosaic pepper plant.	6	6	Do.
Do	1 (healthy)	do	Aphids from healthy pepper plant (control).	6	0	Do.
Oct. 4	3-H (mosaic)	Tobacco	Artificial	10	7	Oct. 12
Do	4 (healthy)	do	Control	10	0	Do.
Do	3-H (mosaic)	Cucumber	Artificial	9	4	Oct. 10
Do	do	do	Aphids from mosaic pepper plant.	9	9	Do.
Do	4 (healthy)	do	Aphids from healthy pepper plant (control).	9	0	Do.
Nov. 7	6-R (mosaic)	Tobacco	Artificial	6	5	Nov. 15
Do	2 (healthy)	do	Control	6	0	Do.
Do	6-R (mosaic)	Cucumber	Artificial	8	4	Do.
Do	2 (healthy)	do	Control	6	0	Do.

Inasmuch as these experiments indicated that cucumber mosaic could be transmitted to tobacco through the use of the pepper as an intermediate host, another series of inoculations was made in which

peppers were inoculated with the expressed juice of mosaic tobacco plants and the inoculations later made from these plants to both cucumber and tobacco. All of these inoculations were made by the artificial method in the case of tobacco, but the cucumber plants in some cases were also inoculated by means of aphids as in the preceding experiments. The results of these experiments (Table XXII) indicate that cucurbit mosaic can be transmitted to tobacco through the pepper, and that tobacco mosaic can be transmitted in similar manner to the cucumber.[5] These results seem to give some support to the belief that the causal agencies of these diseases are identical, but as yet this hypothesis can not be considered as proved.

TABLE XXII.—*Results of cross inoculation of cucumber and tobacco plants from pepper plants infected with tobacco mosaic*

Date of inoculation	Pepper plant used as inoculum	Plant inoculated	Method of inoculation	Number of plants inoculated	Number of plants mosaic	Date observed
Oct. 17, 1922	3-a (mosaic)	Tobacco	Artificial	8	6	Oct. 26, 1922
Do	10 (healthy)	do	Control	8	0	Do.
Do	3-a (mosaic)	Cucumber	Aphids from mosaic pepper plant.	8	5	Do.
Do	10 (healthy)	do	Aphids from healthy pepper plant (control).	8	0	Do.
Nov. 14, 1922	2-c (mosaic)	Tobacco	Artificial	8	5	Nov. 22, 1922
Do	12 (healthy)	do	Control	8	0	Do.
Do	2-c (mosaic)	Cucumber	Artificial	8	5	Nov. 20, 1922
Do	do	do	Aphids from mosaic pepper plant.	8	8	Do.
Do	12 (healthy)	do	Aphids from healthy pepper plant (control).	8	0	Do.
Mar. 23, 1923	4-d (mosaic)	Tobacco	Artificial	8	6	Mar. 31, 1923
Do	7 (healthy)	do	Control	8	0	Do.
Do	4-d (mosaic)	Cucumber	Artificial	10	6	Mar. 30, 1923
Do	do	do	Aphids from mosaic pepper plant.	10	8	Do.
Do	7 (healthy)	do	Aphids from healthy pepper plant (control).	10	0	Do.

CROSS-INOCULATION EXPERIMENTS WITH THE PIGWEED

CROSS INOCULATION FROM MOSAIC PIGWEED TO THE CUCUMBER

Field observations at Madison during the summer of 1920 showed that pigweeds (*Amaranthus retroflexus*) which were growing near mosaic cucumbers were affected with a mosaiclike disease. The affected plants were dwarfed, the leaves were irregular in shape, and the younger leaves were mottled in a manner typical of the mosaic diseases. An examination also showed that these plants were infested with aphids, which were also abundant on the nearby mosaic cucumber plants. Aphids from the supposedly mosaic pigweed were transferred to healthy cucumber plants under cages in the greenhouse, and mosaic symptoms later developed on the majority of these plants. Control plants with aphids from healthy pigweeds, however, remained free from mosaic. In order to eliminate the possibility that some of the aphids on the pigweed had recently come from mosaic cucumber plants and were carrying

[5] Since these experiments were undertaken, O. H. Elmer, of the Iowa Agricultural Experiment Station, has reported that he has secured infection in tobacco plants directly inoculated with cucurbit mosaic. (*15*)

the disease from the cucumber rather than the pigweed on which they were collected, further experiments were undertaken in which mosaic pigweeds were first freed from aphids by spraying and then covered with insect-proof cages. After sufficient time had elapsed to insure the plants being free from insects, aphids were transferred to the pigweed from healthy cucumber plants. At the same time, other aphids from the same healthy cucumber plants were placed on healthy pigweeds under separate cages. Ten days later the aphids on both the healthy and mosaic pigweeds were transferred to separate lots of healthy cucumber plants under cages in the greenhouse. The cucumbers on which the aphids from the mosaic pigweeds were placed developed mosaic in a majority of cases, while the control plants remained healthy. Inoculations were also made by artificial methods from mosaic pigweeds to cucumber plants, both in the field and greenhouse. In these inoculations the crushed leaf tissues of the mosaic plant, inserted in longitudinal incisions made in the stem of the cucumber, were used as inoculum. As shown in Table XXIII, a high percentage of infection resulted from this method as well as from the use of aphids.

TABLE XXIII.—*Cross inoculations from mosaic plants of Amaranthus retroflexus to healthy cucumbers*

Date of inoculation	Method of inoculation	Location	Number of plants inoculated	Number of plants mosaic	Date observed
Aug. 12, 1920	Aphids from mosaic pigweed plant	Greenhouse	6	6	Aug. 22, 1920
Do	Aphids from healthy pigweed plant (control).	do	6	0	Do.
Aug. 13, 1920	Artificial	Field	8	6	Aug. 25, 1920
Do	Control	do	8	0	Do.
Aug. 18, 1920	Aphids from mosaic pigweed plant	do	8	7	Sept. 1, 1920
Do	Aphids from healthy pigweed plant (control).	do	6	0	Do.
Do	Aphids from mosaic pigweed plant	Greenhouse	7	7	Do.
Do	Aphids from healthy pigweed plant (control).	do	7	0	Do.
Do	Artificial	do	8	2	Do.
Do	Control	do	6	0	Do.
Do	Artificial	Field	12	3	Do.
Do	Control	do	8	0	Do.
Sept. 2, 1920	Artificial	do	10	6	Sept. 13, 1920
Do	Control	do	6	0	Do.
Sept. 4, 1920	Artificial	do	6	5	Do.
Do	Control	do	6	0	Do.
Do	Artificial	Greenhouse	4	1	Do.
Do	Control	do	4	0	Do.
July 15, 1922	Aphids from mosaic pigweed plant	do	12	12	July 25, 1922
Do	Aphids from healthy pigweed plant (control).	do	10	0	Do.
June 30, 1923	Artificial	do	6	3	July 7, 1923
Do	Control	do	6	0	Do.

CROSS INOCULATION FROM MOSAIC CUCUMBER PLANTS TO THE PIGWEED

As a result of the successful inoculations from mosaic Amaranthus plants to the cucumber, efforts were made to transmit cucurbit mosaic to this host. Aphids from mosaic cucumber plants were transferred to the leaves of healthy pigweeds growing under cages in the greenhouse, and aphids from healthy cucumber plants were placed on other pigweeds as controls. The inoculated plants had been grown from seed in the greenhouse and were from 6 to 12 inches tall. At the end of 7 days the inoculated plants showed a slight

downward curling of the youngest leaves, and at the end of 12 days a definite mottling began to appear. In the course of the next 2 weeks the mosaic plants developed typical mosaic symptoms and the leaves were mottled and deformed as the new growth developed. The early symptoms of the disease on the pigweed are less marked than with any plants on which the writers have obtained infection, and for this reason the incubation period at first appears to be longer than it really is. As the experiments have been continued it has become evident that the incubation period for this host is approximately the same as for the cucurbits, varying from 7 to 9 days.

As shown in Table XXIV, infection has been accomplished on the pigweed by both methods of inoculation. The artificial inoculations were made in the same manner as those used in inoculating cucumber plants from mosaic pigweeds and a fair amount of infection resulted from this method. The results of these experiments indicate that the pigweed is readily infected with cucurbit mosaic and that such infection occurs under natural conditions in the field. The occurrence of such natural infection has often been observed in fields where pigweeds were growing between rows of cucumbers affected with mosaic. Up to the present the only mosaic pigweeds which have been found have apparently been infected from the cucumber and there is no evidence to indicate that the disease is carried over winter by this host.

TABLE XXIV.—*Cross inoculations from mosaic cucumbers to healthy plants of Amaranthus retroflexus*

Date of inoculation	Method of inoculation	Location	Number of plants inoculated	Number of plants mosaic	Date observed
Apr. 21, 1921	Aphids from mosaic cucumber plant	Greenhouse	1	1	May 9, 1921
July 14, 1922	do	do	4	4	July 31, 1922
Do	Aphids from healthy cucumber plant (control).	do	4	0	Do.
July 19, 1922	Aphids from mosaic cucumber plant	do	8	8	Aug. 1, 1922
Do	Aphids from healthy cucumber plant (control).	do	8	0	Do.
Aug. 2, 1922	Artificial	do	10	6	Aug. 18, 1922
Do	Control	do	10	0	Do.
Aug. 10, 1922	Artificial	do	5	3	Aug. 28, 1922
Do	Control	do	5	0	Do.

SYMPTOMS OF CUCURBIT MOSAIC ON AMARANTHUS RETROFLEXUS

Mosaic Amaranthus plants are much dwarfed and usually reach a height of only 8 to 10 inches, while healthy plants are often 2 to 3 feet tall. The affected plants show a typical mosaic mottling of the foliage, the major portion of the leaf becoming a yellowish-green with smaller dark-green areas scattered over the surface. The petioles of such leaves are usually longer in proportion to the blade than those of healthy plants, and this, together with the shortening of the internodes, gives the mosaic plant an abnormally spreading habit (pl. 3, E). The leaves of mosaic plants are usually smaller, narrower, and more irregular in outline than those of healthy plants. Mosaic plants usually develop only a single stem and do not branch as commonly as do healthy pigweeds. Seed is produced by mosaic plants, but only in small amounts.

Observations have shown that another species of pigweed, *Amaranthus blitoides* Wats., occasionally shows symptoms which have somewhat the appearance of a mosaic disease, but up to the present the writers have not been able to demonstrate that it is transmissible.

Cross-Inoculation Experiments with Plants of other Genera

In the course of the studies on the overwintering of cucurbit mosaic, a number of plants of various families have been found to show symptoms of what appear to be mosaic diseases. Symptoms of this type have been noted on a perennial species of Helianthus in a number of cases, but no infection has been produced on the cucumber from this host, nor has the disease been transmitted to healthy plants of the same species. Symptoms similar to those of mosaic have also been found on *Arctium lappa* L., *Abutilon theophrasti* Medic., *Malva rotundifolia* L., *Oxybaphus nyctagineus* (Michx.) Sweet, *Ambrosia trifida* L., *Bidens vulgata* Greene, and on species of Anemone, Xanthium, and wild Rubus. A limited number of inoculations have been made with all of these plants, but up to the present the writers have no evidence which indicates that these are transmissible mosaic diseases. These experiments are being continued, however, and it may be found that certain of these hosts are affected with mosaic.

Relation of Annual Host Plants to the Overwintering and Dissemination of Cucurbit Mosaic

The annual host plants of cucurbit mosaic would not appear to be concerned in the overwintering of the disease unless the seed of such plants carries the disease from year to year. Experiments have been conducted with seed from annual plants outside the Cucurbitaceae which are known to be susceptible to cucurbit mosaic—pepper, martynia, pigweed, and cultivated Physalis. From 300 to 700 seedlings have been grown from seed of mosaic plants of each of the above hosts, but there has been no evidence that the disease is transmitted through the seed. For this reason, it is felt that the perennial wild hosts are probably the only ones concerned in the overwintering of the disease, with the exception of the wild cucumber.

The annual host plants of cucurbit mosaic may be of importance, however, as sources of infection to the cultivated cucurbits during the growing season. In the course of experiments on the control of cucurbit mosaic through the eradication of the perennial wild host plants, it has been found that the disease may be contracted by annuals from the perennials and carried on the annual plants during the summer after the perennial wild hosts have been destroyed. Field observations have shown this to be true in the case of *Amaranthus retroflexus* and cultivated species of Physalis, and these host plants have apparently acted as sources of infection to adjacent cucurbits throughout the season. It is evident, therefore, that these annual hosts are of practical importance from the standpoint of the control of the disease.

Relation of Wild Host Plants to the Control of Cucurbit Mosaic

As cucurbit mosaic is so readily transmitted by insects in the field, it has proved difficult to check it after it once appears in the fields to any appreciable extent. For this reason, the most prac-

ticable method of controlling the disease has appeared to consist of the early removal of the wild host plants which represent the original sources of infection. Field experiments have been in progress for the past three years in an effort to determine the practical possibilities of controlling the disease by the eradication of these wild hosts and the work is being continued. As a result of this work the host range of cucurbit mosaic has been found to be larger than was at first suspected and the eradication program has consequently been somewhat enlarged. The evidence obtained up to the present indicates definitely that wild host plants are probably responsible for practically all the primary infection on the cultivated cucurbits. The practical possibilities of controlling the disease by the eradication of these host plants have not as yet been fully worked out, but recent results have offered considerable encouragement.

SUMMARY

Continued investigations of the overwintering of cucurbit mosaic have shown that the disease does not persist in the soil.

Further trials with the seed of mosaic cucumber, squash, muskmelon, and pumpkin plants indicate that seed transmission of the disease probably does not occur or is so rare as to be of little significance.

There is no evidence that the striped beetle (*Diabrotica vittata*) is an agency in overwintering cucurbit mosaic.

Cucurbit mosaic is transmissible through the seed of the wild cucumber (*Micrampelis lobata*) and the disease is transmitted from this host to the cultivated cucurbits by the cucumber aphis (*Aphis gossypii*), the striped beetle, (*Diabrotica vittata*), and the 12-spotted beetle (*D. 12-punctata*). The striped beetle is the chief agency in transmitting the primary infection from the wild cucumber to the cultivated cucurbits, as this insect first feeds on the wild host and later migrates to the cultivated cucumbers. Field experiments indicate that the beetles may thus carry infection over distances of at least 400 yards.

Surveys of cucumber-growing districts in Wisconsin and northern Illinois indicate that the wild cucumber is an important factor in overwintering mosaic.

The milkweed (*Asclepias syriaca*) is susceptible to cucurbit mosaic, and is important as a means of overwintering the disease. Mosaic lives over in the roots of the milkweed, and mosaic plants are often found in the vicinity of cucumber fields. The cucumber aphis feeds on the milkweed and acts as a carrier of the disease from this host to the cucumber. Observations indicate that most mosaic milkweeds are originally infected from adjacent cucumbers. In many localities the milkweed appears to be of more importance than the wild cucumber as a source of infection to the cultivated cucurbits, as the mosaic plants usually occur in the immediate vicinity of the fields.

The pokeweed (*Phytolacca decandra*) is susceptible to cucurbit mosaic, and the disease found on pokeweeds in the field is transmissible to the cucumber. Mosaic lives over winter in the roots of the pokeweed, and the cucumber aphis acts as a carrier of the disease from the pokeweed to the cucurbits. Mosaic pokeweeds are rare in

Wisconsin but occur in southern Michigan and northern Indiana. The pokeweed is common in southern Indiana and in the cucumber-growing sections of southern Illinois, where it seems to be an important source of infection to the cucurbits.

Cucurbit mosaic also lives over winter in the roots of catnip (*Nepeta cataria*) and certain perennial species of Physalis.

In cross-inoculation experiments with plants of the Cucurbitaceae, all species tested have proved to be susceptible to mosaic except those of the genus Citrullus. Infection has been produced in 11 genera, including 23 species and 96 horticultural varieties.

Successful cross inoculations have been made from mosaic cucumbers to the *Martynia louisiana*, pigweed (*Amaranthus retroflexus*), and pepper (*Capsicum annuum*).

The pepper is also susceptible to tobacco mosaic, and it has been found that pepper plants may be infected with tobacco mosaic and the disease then transmitted from them to either tobacco or cucumber. Similar results were obtained from pepper plants inoculated with cucurbit mosaic, and the results indicate that the infective principle of tobacco and cucurbit mosaic are intertransmissible.

There is no evidence of seed transmission of mosaic in the case of martynia, pepper, or pigweed, and as these plants are annuals they do not seem to be concerned in overwintering mosaic. These annual hosts, when infected with mosaic, however, often act as sources of infection to cucurbits during the summer.

Experiments on the control of cucurbit mosaic by removal of the wild hosts on which it overwinters are now in progress.

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PRELIMINARY REPORT ON COLLOIDAL CLAYS AS EMULSIFIERS FOR MINERAL OILS USED IN SPRAYING CITRUS GROVES [1]

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INTRODUCTION

Soap emulsions of mineral lubricating oils have been used for many years in Florida for spraying citrus trees for the control of several species of scale insects and white flies. These are usually made according to the following formula:

Mineral oil	gallons	2
Water	do	1
Caustic potash fish oil soap	pounds	2

Several mineral oils have been used, but those used most extensively have the following physical specifications:

Oil	Specific gravity at 27° C.	Flash point (° C.)	Fire point (° C.)	Viscosity [1]	Volatility [2]
Medium	0.886	184	207	365.3	4.9
Heavy	.896	163	245	1,121.0	.16

[1] Engler, $H_2O=100$. [2] 1 gram for 4 hours at 105° C.

The materials are put into a kettle or other vessel that will stand fire and are heated to the boiling point—about 168° F. After removal from the fire, but while still very hot, the mixture is pumped with a bucket pump from the kettle to another receptacle, and back again. The formula just mentioned is sufficient for 197 gallons of water, making about 1 per cent of oil in the diluted spray material.

These soap-mineral oil emulsions have given most satisfactory results from the standpoint of insect control. The simple soap emulsions, however, have certain limitations which make them somewhat inconvenient for citrus growers. For example, they are not sufficiently stable to mix with "hard" or deep-well water unless this water is first treated with chemicals to "soften" it; neither will they mix with lime-sulphur solution without the emulsions themselves being specially treated with a stabilizing agent. They will, however, mix with alkaline Bordeaux mixture in all proportions.

The simple soap emulsions can be made to mix with any "hard" or deep-well water found in Florida citrus groves or with lime-sulphur solution by means of stabilizers such as glue, casein, milk powder, flour, corn starch, laundry starch, and water glass. When these are placed in the emulsions immediately before they are to be used satisfactory results have always followed; but if the organic materials are put into the emulsions any considerable length of time before they are to be used fermentation usually takes place and the entire emul-

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sion breaks down, resulting in total loss, because it can not be re-emulsified. Except for these defects the soap emulsions have given great satisfaction.

In the search for a nonfermentable stabilizer for soap emulsion experiments were made with a number of inorganic materials, including kaolin, a china clay composed mostly of hydrous aluminum silicate, which did not prove satisfactory for this purpose. Following suggestions of the junior author, these experiments led to the use of it as an emulsifier instead of soap.

KAOLIN AS AN EMULSIFIER

The first attempt by the writers to use the kaolin as an emulsifier resulted in obtaining a perfect emulsion, and thereafter practically all the experimental work was directed to determining the quantity of kaolin required for best results. Oils having the same physical specifications as those previously given were used in all the kaolin emulsion experiments.

The following formulae were tried in preparing the first few lots of kaolin emulsion:

	Mineral lubricating oil	Water	Kaolin
	Gallons	*Gallon*	*Pounds*
A	2	1	4
B	2	1	2⅔
C	2	1	2

PROCEDURE.—Milk of kaolin was made by simply adding the water to the kaolin and permitting the mixture to stand 15 to 30 minutes. The oil was then added to this mixture and emulsified without the aid of heat by passing the materials twice through a bucket pump.

All of the above formulae produced reasonably satisfactory emulsions. The first test, where 4 pounds of kaolin were used, produced a perfect emulsion, but the resulting paste was entirely too stiff for practical grove spraying. Where only 2 pounds were used, the emulsion tested satisfactorily when diluted with water, but there appeared to be some free oil in the emulsion itself, and it was thought advisable to use a little more kaolin. The formula whereby 2⅔ pounds were used, or 1⅓ pounds per gallon of oil, proved to be perhaps the most satisfactory, and it was used in practically all extensive spraying work.

Attempts were made to produce an emulsion by only stirring, in much the same manner as in preparing the cold-stirred soap emulsions. The oil was added very slowly and gradually to the milk of kaolin, but the writers were not able to stir it violently enough to produce a satisfactory emulsion. No doubt an emulsion can be made by this method if sufficient stirring is given the mixture and the oil is added very gradually.

FULLER'S EARTH AS AN EMULSIFIER

Fuller's earth, a crude material of variable composition, but mostly of hydrous aluminum silicate, used extensively in the textile and oil industries, was tested for its value as an emulsifying agent for mineral oils. Experiments were conducted to determine whether it is a satisfactory material for emulsifying mineral oil, the following formula being used:

Mineral lubricating oil	gallon	1
Water	do	1
Fuller's earth	pounds	2⅔

As was the case with kaolin, fuller's earth dispersed readily in the water, after which the oil was added. The entire mixture was pumped three times, and the resulting emulsion proved satisfactory, but it was much thinner than where a similar proportion of kaolin was used. In order to test fuller's earth further, the following formula was tried:

Mineral lubricating oil	gallons	2
Water	do	1
Fuller's earth	pounds	2⅔

The entire mixture was pumped twice, resulting in a very thick emulsion, which when tested with water gave a little free oil, but further trials gave entirely satisfactory results.

In laboratory tests kaolin and fuller's-earth emulsions were mixed with practically all spray materials used in citrus groves, including 1–1–50, 3–3–50, 5–5–50, neutral and improperly prepared Bordeaux mixtures as well as "hard" deep-well water, and many other solutions. Concentrated sulphuric, nitric, and hydrochloric acids, saturated caustic soda and concentrated lime-sulphur solutions had no appreciable effect on causing the oil to separate.

These emulsions do not separate into layers within a reasonable length of time as do the soap emulsions and commercial miscible oils, nor do they deteriorate rapidly. Kaolin emulsion has been kept in an open barrel for more than three months during the hottest period of the year and it still remained satisfactory for use. Certain changes, however, take place. With age the emulsion changes from a creamy yellow to a bluish color beneath the surface and an offensive sulphurous odor is given off. One lot separated and spoiled after remaining in an open barrel a year, and efforts to reemulsify it by adding water and more kaolin were not successful.

BRICK CLAY AS AN EMULSIFIER

Brick clay was also tested and found to make a perfect emulsion, but because of the presence of more or less sand in the clay it probably would not be a satisfactory emulsifier for spraying emulsions.

In addition to the above-mentioned colloidal clays, hydrated lime and commercial Bordeaux pastes and powders were used, tested in the laboratory only, and found to be reasonably satisfactory but not nearly as good as kaolin or fuller's earth.

KAOLIN LIME-SULPHUR OIL EMULSION

After several unsuccessful attempts, kaolin also proved to be a satisfactory emulsifier to convert lime-sulphur solution and oil into an emulsion. The failures were due to the allowance of insufficient time for the kaolin to become completely dispersed by the lime-sulphur solution. It was thought advisable to emulsify the regular quantities of materials usually recommended for 150 gallons of standard dilution. They are as follows:

Mineral lubricating oil	gallons	1
Lime-sulphur solution	do	3
Kaolin	pounds	2

When put in the lime-sulphur solution the kaolin did not disperse as quickly as in water, but on standing overnight it went into suspension readily. The oil was added and emulsified with a bucket pump, a satisfactory product resulting.

Fuller's earth was used instead of kaolin to make the following excellent emulsion:

To 3 quarts of lime-sulphur solution 1 pound of fuller's earth was added. The clay formed a suspension very readily in the lime-sulphur solution, after which 3 quarts of oil was added and the materials emulsified by pumping twice.

FIELD EXPERIMENTS

On February 9, 1923, some of the kaolin emulsion was used for experimental spraying on one lemon tree in full bloom and with much new growth, two orange trees, and two wild cherry trees (*Prunus serotina*) in full flush of growth. Six days later the sprayed trees were examined. The cherry trees were quite badly defoliated, but the only damage observed on the citrus trees was the slight curling of six leaves on the lemon. No excessive defoliation had taken place.

Normally, a few of the oldest leaves fall after applications of the oil sprays in general use. Leaf droppage induced by oil sprays seldom if ever occurs until the third day after the spray is applied. The distinctive features of this type of defoliation are as follows: The leaf retains its normal green color, and a few days after the spray is applied the blade, while still green, disjoints and falls from the petiole, leaving it attached to the branch. If the dosage is excessively strong, or if two or more applications of normal dilution are made within two or three weeks without rains to remove the excess oil, or if the emulsion breaks, severe defoliation and fruit droppage is likely to occur.

On February 27, 1923, the same trees that were sprayed 18 days previously were sprayed again, using the kaolin emulsion 1 to 50, or approximately 1½ per cent of oil in the diluted spray. For this work 1½ pounds of kaolin per gallon of oil were used instead of the 1 pound, as of February 9. The material mixed well, but the trees seemed to have a greasy appearance after being sprayed, and the drops of spray were quite large. There were blooms on both the lemon and orange trees on the date of spraying. It rained about 5 hours after the spray had been applied, and also on the following day. Little or no damage was done to these trees.

On February 13, 1923, the following sprays were applied on bearing orange trees:

3 quarts simple soap emulsion in 50 gallons of water.
3 quarts of kaolin emulsion in 50 gallons of water.
3 quarts of kaolin emulsion in 50 gallons of 3–3–50 Bordeaux mixture.
3 quarts of kaolin emulsion and 2 pounds of soda-sulphur in 50 gallons of water.
3 quarts of kaolin emulsion and 5 quarts of lime-sulphur solution in 50 gallons of water.

At this time ripe fruit was on the trees, a few tiny bloom buds (not full white) were present, and there was considerable new growth. Two days later all of the trees were carefully examined to determine if any damage had been done. No damage whatever was observed. The fruit, new leaves, and tiny buds remained normal, and there was no excessive shedding of old leaves.

Again, on May 11, 1923, 13 seedling orange and grapefruit trees near Orlando, Fla., were sprayed with kaolin emulsion at the rate of 9 quarts to 150 gallons of water, or approximately 1 per cent oil in the diluted spray. The grapefruits were nearly 1½ inches in diameter, and the oranges about 1⅛ inches. The sun was bright and the temperature approximately 80° F. The emulsion mixed perfectly with the lake water and there was no residue in the tank when the load was finished. The spray collected in large drops on the fruit and foliage and they appeared to be covered with free oil. A few minutes after spraying spots (commonly called "shadows") with an oil-soaked appearance developed where the drops of spray had collected. Some of the spots caused by the oil had not left the fruit as late as August 1. Such spotting frequently occurs on citrus following oil sprays, but if not too severe it usually disappears long before the fruit takes on the color of maturity.

Extensive field tests were made with kaolin emulsion through the entire season of 1923. Many thousand gallons were used in combination with various strengths of Bordeaux mixture during the scab and melanose spraying seasons, and in every case equally satisfactory results were obtained as with soap emulsion, and no injury followed. It was also used extensively in combination with lime-sulphur solution and dry lime sulphur during late June and early July to kill scale insects and rust mites following Bordeaux oil emulsion applied for scab or melanose control.

As a general thing it gave satisfactory results in controlling scale insects and rust mites, but in some cases considerable injury followed. The kaolin emulsion alone has never caused any damage different in appearance or extent from that following soap emulsion applied under similar conditions, and in only a few instances has lime-sulphur solution caused damage. There seems to be a greater liability to damage when an emulsion is combined with lime-sulphur solution than when the two are applied separately.

Fuller's earth was used with excellent results by a large fruit company on several acres of oranges and grapefruit during 1923 and on about 400 acres during 1924. It was used with "hard" or "sulphur" water from deep wells, and it mixed better than any emulsion ever before used by this company. They used a heavy oil diluted to 1⅓ per cent and got some oil burn. The oil concentration was reduced to 1 per cent and still some burn developed. They then added 2 pounds of hydrated lime to each 50 gallons of diluted spray and thereby practically eliminated the spray injury.

KAOLIN AND FULLER'S EARTH EMULSIONS WITH CALCIUM CASEINATE

Both kaolin and fuller's-earth emulsions when applied on citrus foliage have the appearance of not spreading very well, but, as will be shown later, this appearance is entirely deceptive. To overcome this apparent objection, several experiments with calcium caseinate were tried. On May 23, 1923, six orange trees were sprayed, using kaolin emulsion 1⅓ per cent in the diluted spray and calcium caseinate at the rate of 1½ pounds to each 50 gallons of water. This spray spread over the leaves and fruit uniformly and did not collect in drops on the fruit except on the lower side. On August 27 two 50-gallon tanks of kaolin emulsion and two tanks of fuller's-earth

emulsion were applied on budded orange trees. To one tank of each was added 8 ounces of calcium caseinate, one load of each not having this material. The calcium caseinate made the spray spread better when it was being applied and it was a much smoother material; but on the following day there could be observed no difference in the way the respective sprays had spread and dried on the foliage. Apparently there was just as even a film of oil on one plot as on the other. From this test there is some question whether it is profitable to add calcium caseinate to kaolin emulsion.

KAOLIN AND FULLER'S EARTH LIME-SULPHUR OIL EMULSION

Kaolin lime-sulphur oil emulsion was used on August 27 to spray some young orange trees, at the rate of 1½ gallons to each 50 gallons of spray. This gave a 1–50 lime-sulphur solution and a 1 per cent oil, the normal dilution for both when applied separately. These three 50-gallon loads mixed very satisfactorily and came out of the machine nicely. The spray seemed to spread over the fruit and foliage evenly, but did not have the smooth appearance of the soap emulsions. No injury was apparent 24 hours after spraying, but 8 days later it was observed that considerable shedding of foliage had taken place. The foliage shed, however, consisted mainly of old leaves. Some fruit also was damaged on the upper surfaces. At first this appeared to be excessive, but it is doubtful that more than 50 fruits were damaged on the entire plot which received the 150 gallons of material. The injury, however, was greater than good grove practice would allow.

Two loads of 50 gallons each of fuller's earth lime-sulphur oil emulsion were sprayed on oranges on August 27, 1923, one with and one without the addition of calcium caseinate. This made the dilutions of the lime-sulphur and oil emulsion the same as is frequently used in grove practice.

No injury was noticed at the expiration of 24 hours, but 8 days after spraying defoliation was very severe, much of the fruit had been burned on the side toward the sun and many limbs one-half inch in diameter and smaller were dead. The injury resembled that which usually follows applications of sulphonated oil on citrus trees and fruit. After such severe injury it was thought best to discontinue further work on this formula. Fuller's earth and kaolin emulsion (made with water instead of lime-sulphur solution) were used on the same day without any injurious results.

DIRECTIONS FOR MAKING KAOLIN OR FULLER'S EMULSION IN LARGE QUANTITIES

To make 3 barrels of emulsion put 50 gallons of water in the clean tank of a power sprayer and add 133 pounds of kaolin or fuller's earth to the water; let the water act upon the clay for one-half hour, or until it is completely saturated. Up to this point no stirring whatever should be done, as stirring retards complete dispersion of the kaolin by forming an apparently impervious coating around the larger lumps. Add 100 gallons of oil of the desired consistency. As soon as the clay is completely dispersed start the agitator and pump. The mixture should be pumped into another container and then pumped back again, or pumped directly back into the original tank

until the emulsion becomes thick, when it may be pumped into storage barrels. A good emulsion looks much like a firm mayonnaise salad dressing.

This emulsion has been made many times in 50-gallon and several times in 150-gallon lots by running the mixture twice through the pump of a power spraying outfit.

SUMMARY

Kaolin, fuller's earth, and other colloidal clays may be used in lieu of soap in making emulsions of mineral lubricating oils for spraying citrus trees. These emulsions are about as effective against insect pests as are the soap emulsions, and no more likely to cause spray burn. The main value, perhaps, in these clay emulsions is that they will mix with any water or desired spray combination without special treatment, are easily made, keep indefinitely, and cost much less than soap emulsion, both for material and for labor. The kaolin used for the emulsion should not cost more than from one-sixth to one-ninth as much as the soap, and fuller's earth is cheaper than kaolin. The principal objection to these emulsions is that they are a semipaste rather than a fluid.

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RELATION BETWEEN CERTAIN HERITABLE PROPERTIES OF WHEAT AND THEIR CAPACITY TO INCREASE PROTEIN CONTENT OF GRAIN[1]

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Table I gives the relation of two heritable characters of wheat with the percentage of increase in protein of the grain produced by an application of nitrogen supplied relatively late in the growth period of the plants. Explanation of the values of the data is as follows:

Column A gives the maximum per cent of gain in protein assumed to be due to application of nitrogen to the plant. The values were obtained by dividing the per cent protein in the grain of cultures that received nitrogen when 90 days old by that contained in the cultures which produced the lowest per cent of protein. The lowest values were obtained either from the cultures that did not receive nitrogen or those that received it at the time of planting. (That nitrogen supplied to wheat in pot cultures, or in the field at the time of planting does, in some cases, result in a product lower in protein than would obtain if this material were not supplied, has been observed by some other investigators. As the explanation for this phenomenon would be aside the subject matter of this article, it will not be considered in this paper.)

Column B gives the per cent grain to total dry matter. These values represent differences considered as heritable among the varieties. (While the characters in columns B and C are considered by agronomists to be heritable and represent distinctions among varieties, it is known that the values of these characters may be changed by environmental conditions. Varieties showing marked differentiations, however, usually maintain approximately their relative order for these characters even under diverse conditions. However, it is necessary, in a study of this kind, to appreciate that the relative values of any of the characters may change.[2])

Column C represents the relative capacity of the varieties to ripen after nitrogen was applied, which factor may also be considered as genetic. The values were obtained by dividing the number of days required for ripening of plants which did not receive nitrogen by the number of days before ripening that nitrogen was applied to the cultures which produced the grain that had the highest per cent protein.

Column B differs from column A in that the order for Sonora and Fulcaster for third and fourth places, and for Hard Federation and Dart's Imperial for fifth and sixth places, are interchanged. When columns A and C are compared, it is to be noted that the varieties for fifth and seventh places are interchanged.

[1] Received for publication Sept. 19, 1924; issued September, 1925.

[2] GERICKE, W. F. SOME EFFECTS OF PHYSIOLOGICAL CONDITIONS ON GENETIC CHARACTERS OF WHEAT Amer. Jour. Bot. 10: 275-277. 1923.

TABLE I.—*Relation between two heritable properties of wheat varieties and their capacity to increase the protein content of grain*

A		B		C	
Capacity to increase in protein		Ratio of grain to total weight		Capacity to ripen after treatment	
Bunyip	1.738	Bunyip	44.5	Bunyip	3.00
Cedar	1.635	Cedar	40.5	Cedar	2.76
Fulcaster	1.625	Sonora	38.9	Fulcaster	2.36
Sonora	1.576	Fulcaster	37.7	Sonora	2.29
Dart's Imperial	1.559	Hard Federation	35.1	Early Baart	2.22
Hard Federation	1.500	Dart's Imperial	32.8	Hard Federation	2.21
Early Baart	1.498	Early Baart	32.7	Dart's Imperial	2.06
White Australian	1.395	White Australian	32.6	White Australian	1.95
Marquis [a]	1.350	Marquis	30.2	Marquis	1.88

[a] For some reason, Marquis did not grow well. There was considerable mortality among the cultures. It is probable, therefore, that the order given it does not indicate its true value among these varieties.

The way these varieties (which represent a range of very early to very late wheats) fall in order of correlation at once suggests causative relations in some of their heritable properties and in the capacity of wheat varieties to produce high-protein grain. Owing to the fact that the protein content of grain can be increased by merely supplying nitrogen to the plants at certain phases of their growth, it follows that the genetic characters of varieties which are correlated to the capacity of the plants to increase the protein content of the grain must be agencies which influenced the physiological processes determining the protein content of wheat. As this factor obviously bears on the relative rate of intake of nitrogen to that of other processes of growth, it follows that the effect of these genetic properties on the protein content of wheat must influence the rate of absorption and utilization of nitrogen by the plants.

The ratio of grain to total dry matter is an expression of the relative power of varieties to utilize absorbed material, including nitrogen, for grain production. However, as the per cent of protein in grain of any variety is determined by the amount of nitrogen those plants utilize for grain, and by the amount of grain produced, it follows that any change in this value must be due to changes in either or both factors. Whether wheat varieties which have the genetic property to produce high values of grain to total dry weight also possess correspondingly great capacity to produce high-protein grain when physiological conditions supervene which make this possible, has apparently not been determined. The fact of correlation between these properties, as shown by the data, is evidence that varieties which have great efficiency for production of grain to total dry weight likewise have correspondingly great capacity to augment the protein content of the grain above the minimum for that variety. This, of course, does not mean that those varieties characterized by high percentage of grain to total dry weight invariably produce higher protein grain per se than do those varieties that do not have this genetic feature. The ratio of grain to total dry matter is only a measure of the capacity of the plants to increase the nitrogen in the grain and can not by itself determine what the absolute protein content of the grain will be. Furthermore, this property is also an expression of the degree of fluctuation to which the variety is subject.

Whether or not high-protein grain will result from the capacity of any variety of wheat to produce high percentage grain to total

dry weight, granted that the proper physiological conditions prevail, depends largely on what the minimum for that variety happens to be. If, for example, two different varieties each having the same percentage of grain to total dry weight, and, consequently, equal capacity to increase the protein content of their grain, having 6 and 8 per cent protein, respectively, as the minimum, would, as a result of treatment, produce grain that would vary proportionally in protein content. But the increase in one case, say it was 50 per cent, would be such as to retain for that product the character of soft wheat, while equal per cent increase in the other variety would result in grain belonging to the hard wheat class; 9 and 12 per cent, respectively, would be the per cent protein of these two varieties.

In the correlation between the order of ripening of the varieties after nitrogen was supplied, and that of the gain in per cent protein of the grain, is evidence that the genetic property which determines how quickly any wheat variety matures likewise expresses the relative capacity of that variety to increase the protein in grain. Whether any variety produces high-protein grain is, however, not determined by the mere property of earliness but by the physiological condition already defined, namely, the relative rate of the absorption of nitrogen, which, in turn, is dependent on the supply available to the plants. Thus if varieties requiring different numbers of days are to absorb and utilize a given limited amount of nitrogen supplied to the culture 90 days after planting, it is obvious that those varieties which utilize this material in the least number of days have a higher rate for that process, or processes, than have varieties which require a greater number of days. It is in this relation that the genetic property of earliness bears on the per cent protein in grain. It affects the relative rate of intake of nitrogen by the plants. Therefore, wheats that are early by heritable property are physiologically better adapted to produce higher-protein grain than are late wheats. This, however, in turn, does not mean that late varieties are necessarily low-protein wheats. On the contrary, many are relatively high in protein. If sufficient nitrogen is available to the plants for a period of three to six weeks during certain phases of their growth (differing with the varieties), which includes the latter part of the "shooting" period for the culms, the period of heading, and the early part of the period of filling of the mold, then high-protein grain obtains. On the other hand, even though the soil does not contain as much nitrogen as the plants could absorb (conditions that usually prevail in wheat-growing sections), this does not necessarily infer the production of low-protein wheat. Other compensating factors, such as that related to ratio of grain to total dry weight, can preclude the performance of results of any specific factor.

As the protein content of wheat can be markedly affected by manipulation of the supply of nitrogen in the growth media, so climate, season, and soil, through their influence on the rate of supply of nitrogen to the plants must affect the protein content of the grain. In some regions climate and seasonal changes on varietal characters will have more pronounced influence on the quality of grain than in other sections. Likewise the effect of properties of soil on the protein content of grain may be markedly altered by the influence of climate and season. It therefore follows that while the

rate of supply of nitrogen to the growing plants determines the protein content of grain, nevertheless many factors may affect the rate of supply of nitrogen to the plants. Thus the methods of culture to be employed which will effect the desired end will vary with, and depend on, conditions that prevail in wheat-growing areas. Undoubtedly the quality of wheat could be markedly improved in many places if cultural methods were used that would provide the production of sufficient nitrogen during a portion of the latter part of the growth period of the plants. Application of nitrate to partially matured plants in fields where the grain is usually low in protein is, in most cases, impractical. Furthermore, success of this method would be conditioned by ample rainfall to make the salt available, and this is usually precluded by the character of the season.

Thus it appears that the most promising method would be one that would utilize the character of soil and particularly that fraction which is organic matter to act both as a storehouse for and a regulator of the supply of available nitrogen. Certain methods of soil management and crop rotation could be instituted that would undoubtedly affect the rate of supply of nitrogen for any particular year in which wheat is to be grown on any given piece of land. Manures which nitrify relatively slowly presumably could be used to advantage in the production of high-protein wheat.

But aside from the improvement of the quality of wheat directly through nutrition, methods of selection and breeding for high-protein varieties undoubtedly must be continually employed. However, because of the failure to recognize, first, the underlying physiological cause for differences and variation in the protein content of wheat, and second, the relation of genetic characters to the physiological cause, the method for improvement of wheat by selection has been rendered quite tedious. It has kept workers in the dark as to the essential factors that determine the desired results. It is, therefore, thought that the relationships here shown to exist between certain heritable characters of varieties and their capacity to produce high-protein grain will be helpful to plant breeders and agronomists in obtaining such varieties of wheats as will produce high-protein grain even under relatively unfavorable soil and climatic conditions.

SPREADERS FOR SPRAY MATERIALS, AND THE RELATION OF SURFACE TENSION OF SOLUTIONS TO THEIR SPREADING QUALITIES [1]

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INTRODUCTION

For many years efforts have been made to improve the spreading and adhering qualities of spray materials by the addition of some harmless substance that would cause the spray to form a continuous, even film over the plant surface covered. At present our greatest need in orchard practice, aside from the development of certain more effective sprays, is to overcome the difficulty attending the application of sprays in which the surface can not properly and easily be covered.

The continual increase in severity of infestation and the appearance of some insect pest or fungous disease into a new section of the country emphasizes the necessity of either heavier and more careful applications of the poison whereby all of the plant surface will be covered, or the use of a more effective spray. Since most of our important sprays are effective to the extent that fair control has been obtained with them when care is exercised to obtain complete coverage, it is obvious that the main factor contributing to the ravages caused by insect pests and fungous diseases is the inability to cover with comparative ease the entire surface. If the addition of a spreader to a spray improves the covering and assists in more easily obtaining a protective coating of the poison on those surfaces to which the sprays adhere with difficulty, then an important advance has been made in spraying.

An ideal spreader should cause a continuous film of the poison to be deposited on the surface covered instead of collecting in drops, it should increase adherence, it should not react chemically with the poison to form by-products that would cause foliage injury, it should not diminish the toxic properties of the poison, and it should not cost too much. The difficulty in selecting a spreader with these requirements is increased by the fact that the plant may have several different kinds of surfaces. For example, the apple tree has the smooth upper leaf surface, the hairy under leaf surface, and the waxy surface of the partially developed fruit; and in addition it may have the smooth bark and the wrinkled bark of spurs and the young and old leaves which may offer resistance in various ways to the spray. Ruth and Kelley [2] state that the behavior of the surfaces toward sprays changes very rapidly, in some cases markedly within a week or 10 days. It may be impossible to find a spreader that will fulfill all of the requirements. Investigations, however, may reveal materials or combinations of materials that will prove beneficial on certain plants at different periods of growth.

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[1] Received for publication Aug. 24, 1924; issued September, 1925.

[2] RUTH, W. A., and KELLEY, V. W. RECENT ADVANCES IN SPRAYING. Unpublished data. 1922.

The present investigation was undertaken (1) to make a study of the known spreader materials and to search for others that may be more practical; (2) to correlate the surface-tension values of various materials with their spreading properties, and to learn more regarding the fundamental causes of spreading; and (3) to devise a laboratory method whereby the approximate spreading properties of a substance may be estimated.

REVIEW OF LITERATURE

Previous investigations on the spreading properties of different substances consider a wide variety of materials. A very complete bibliography of the literature is given by Moore (*7*)[3] in his general discussion of the subject, and the review will not be repeated here. Summarized briefly, previous investigations have shown that various colloidal substances are most effective in modifying the spray mixture to make it spread over various surfaces and form a continuous film on the objects sprayed. Among those colloids, casein, soap, glue, saponin, and flour have been found adaptable under certain conditions. Other organic materials such as creosote, eugenal, and cresylic acid are reported as having spreading properties on waxy surfaces, but their high cost and other objectionable features make them impracticable for use.

The theory of spreading as applied to spray materials is presented in recent publications by Cooper and Nuttall (*1*) and by Moore (*7*). They elaborate upon the principles of wetting or spreading as first established by Quincke (*8*) in 1877. In a study of the capillary rise of liquids in tubes of different materials the latter formulated the conditions necessary for wetting or spreading to occur. These conditions as applied to spreading provided that in order to state definitely whether a liquid will wet or spread on a solid (or another liquid) it is necessary to know the values for the surface tension of the liquid and of the solid and the interfacial tension of the liquid to the solid. Spreading of a certain liquid or spreader solution would then occur if the surface tension of the solid or surface sprayed is greater than the sum of the surface tension of the liquid and the interfacial tension of the liquid to the solid. The magnitude of the inequality would indicate good or poor spreading of the liquid on the solid. Since, however, the surface tension of the solid—if a solid has surface tension—is indeterminate, it would be impossible to obtain the actual values necessary to ascertain whether a substance would be a good spreader for a specific surface, as, for instance, the apple leaf or fruit. If approximate values could be obtained by substituting a liquid for the solid, as, for example, an oil for the waxy surface of fruit or leaf, the probable spreading properties of a given substance might be estimated. This was accomplished successfully by Cooper and Nuttall (*1*) in their evaluation of the wetting power of various stock dips. Reference will be made to this procedure later.

From the preceding it is apparent that any spray solution to spread well should have both a low surface tension and a low interfacial tension to the solid or surface sprayed, as the sum of these values must be less than the surface tension of the surface sprayed. If a

[3] Reference is made by numbers (italic) to "Literature cited," p. 81

spray does not possess these requirements it may be modified by the addition of some substance that reduces the tension values. The question then arises, what substances will reduce the surface tension of a solution. It is well known that adsorption of a solution at the surface layer will cause a reduction in the surface tension. The same is true regarding adsorption at the interface of a liquid and solid. It then becomes a search for those substances that are adsorbed at the liquid-air interface and the liquid-solid interface. Moore (*7*), in answer to the question regarding those substances that are positively adsorbed in the surface layer or at the interface of a liquid and solid, applies the theory of orientation of molecules at the surface or interface. From the work of Harkins, Brown, and Davies (*3*), of Harkins, Davies, and Clark (*4*), and of Langmuir (*6*), Moore suggests that those substances that contain active polar groups which render them soluble in water and inactive groups that cause adsorption at the surface and thus reduce the surface tension should augment the spreading properties of the solute. Then if the inactive group is similar to the surface (of the plant) to be covered or soluble in it, spreading should result.

Since the amount of adsorption in the surface layer of a solute and at the liquid-solid interface indicates, to a certain extent, the spreading power of the solution, a measurement of the surface and the interfacial tension values should disclose its possibilities as a spreader.

EXPERIMENTAL DATA

In order to obtain more complete data on surface adsorption of various materials, surface-tension determinations of numerous substances were made. The method employed was mechanical and rapid. The Du Nous instrument was used at first, but a new apparatus devised by Fahrenwald (*2*) was found more adaptable for the work at hand. This apparatus measures the surface tension of a solution by the film method. An accuracy of 0.1 dyne-cm. is claimed for the instrument.

All measurements were made at 25° C. The time necessary for static equilibrium to be established was found to vary appreciably with the different substances. In order to proximate the time it takes for a drop of spray to leave the nozzle of the spray gun and to settle on the surface of the plant, about three minutes were allowed for the development of partial static tension before the measurement was recorded. The results given in Table I report the observations made on part of the miscellaneous substances studied.

In order to correlate the surface-tension results with the actual spreading qualities of a substance, several kinds of leaves and half-grown apples were sprayed with similar solutions on which surface-tension observation had been made. The leaves and fruit were sprayed with an atomizer, and the degree of spreading as observed was recorded. The degree of spreading designated by the term "good" indicates the formation of an even film covering the entire surface, "medium" signifies that small portions of the surface drained dry, "poor" indicates very slight spreading, and "none" means spreading on the surface similar to distilled water. The degree of spreading of various substances dissolved or suspended in distilled water at the concentration indicated is given in Table II.

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TABLE I.—*Surface tension of miscellaneous substances in solution*

Material	Concentration	Surface tension	Material	Concentration	Surface tension
	Per cent	*Dynes per cm.*		*Per cent*	*Dynes per cm.*
Water		72.8	Tannin	1.0	48.4
Sodium palmitate	0.1	31.2	Do	.1	57.9
Do	.01	40.5	Do	.01	71.5
Do	.001	50.7	Gum arabic	1.0	72.0
Sodium stearate	.1	34.6	Do	.1	72.1
Do	.01	39.4	Do	.01	72.1
Do	.001	51.0	Glycerine	1.0	71.5
Sodium oleate	.1	24.0	Do	.1	71.5
Do	.01	31.7	Creosote (meta)	1.0	48.0
Do	.001	49.0	Do	.1	69.5
Potassium oleate	.1	25.1	Creosote (beechwood)	.5	43.6
Do	.01	32.0	Do	.05	67.2
Do	.001	54.5	Cresol	1.0	41.0
Soap, Castile	.1	23.2	Do	.1	49.5
Do	.01	31.9	Resorcin	1.0	55.9
Soap, Ivory	.1	25.6	Do	.1	69.2
Do	.01	35.4	Phloroglucine	1.0	62.9
Do	.001	48.3	Do	.1	71.5
Soap, linseed oil (jell)	.1	26.7	Guaiscol	1.0	45.6
Do	.01	37.8	Do	.1	70.0
Do	.001	49.5	Pyrogallol	1.0	70.8
Mineral oil emulsion No. 1	4.0	26.9	Do	.1	71.4
Do	2.0	33.1	Hydroquinine	1.0	71.5
Do	.1	50.6	Oil of cloves (in 10 per cent alcohol)	1.0	39.4
Mineral oil emulsion No. 2	4.0	38.1	Do	.1	44.2
Do	2.0	42.3	Oil of Eucalyptus (in 10 per cent alcohol)	1.0	32.0
Do	.1	55.9	Do	.1	44.3
Casein (in saturated $Ca(OH)_2$)	1.0	51.4	Oil of cedar	.1	42.1
Do	.1	51.7	Oil of cedar (in 10 per cent alcohol)	.1	42.1
Do	.01	53.8	Oil of cottonseed	.1	47.4
Casein (plus 1 per cent $Ca(OH)_2$)	1.0	51.4	Sodium oleate 0.01 per cent + casein ($NaHCO_3$)	.01	30.4
Do	.1	52.0	Sodium oleate + 0.001 per cent casein	.001	51.3
Do	.01	54.0	Sodium oleate 0.01 per cent + casein	.01	.338
Casein (plus 0.1 per cent $NaHCO_3$)	1.0	43.0	Sodium oleate 0.001 + casein ($NaHCO_3$)	.001	50.0
Do	.1	47.2	Oil of cloves 0.1 per cent + sodium oleate	.01	37.8
Do	.01	49.8	Oil of cloves 0.1 per cent + gylcerin	.1	44.3
Milk, skim (on dry basis)	.1	48.0	Oil of cottonseed 0.1 per cent + sodium oleate	.01	38.7
Do	.01	50.1			
Gelatin (purified)	1.0	51.3			
Do	.1	56.5			
Do	.01	62.2			
Glue (commercial)	1.0	46.0			
Do	.1	56.2			
Do	.01	64.8			
Saponin bark	1.0	41.3			
Do	.1	41.6			
Do	.03	50.0			

A comparison of the surface-tension values of the different substances in solution with their spreading qualities, shows that there is no consistent relation between them. Those substances that spread well have a much lower-surface tension than water, but other solutions having even lower values do not show as good spreading qualities. For example, a 0.01 per cent solution of casein, albumin, or saponin spreads well on all of the surfaces tested. On the other hand, the soaps at 0.01 per cent concentration did not spread any better than water, although the surface tension was far below the values for casein, albumin, or saponin. It is evident from these results that there are other influencing factors besides surface adsorption that affect the spreading qualities of a solution.

The other variable—namely, the tension at the interface of liquid-solid—is indeterminate. However, it was thought that by substituting an oil for the solid, as suggested by Cooper and Nuttall, (*1*) and making surface and interfacial determinations, that some interesting data would be obtained. Accordingly, a mineral oil having a specific

TABLE II.—*The spreading properties of various substances on fruit-tree leaves and apple fruit*

Substance	Concentration	Apple leaves	Pear leaves	Prune leaves	Apple fruit
	Per cent				
Sodium palmitate	1.0	Good	Good	Good	Good.
Do	.1	Medium	Poor	Poor	Medium.
Do	.01	None	None	None	None.
Sodium stearate	1.0	Good	Good	Good	Good.
Do	.1	Medium	Poor	Poor	Medium.
Do	.01	None	None	None	None.
Sodium oleate	1.0	Poor	do	Poor	Poor.
Do	.1	None	do	None	None.
Do	.01	do	do	do	Do.
Potassium oleate	1.0	Poor	do	Poor	Poor.
Do	.1	None	do	None	None.
Do	.01	do	do	do	Do.
Soap, Ivory	1.0	Good	Good	Good	Good.
Do	.1	None	None	None	None.
Linseed-oil soap	.1	Medium	Poor	Medium	Poor.
Casein (in $Ca(OH)_2$ sol.)	.01	Good	Good	Good	Good.
Casein (in $NaHCO_3$)	.01	do	do	do	Do.
Milk, skim (fresh)	1.0	do	do	do	Do.
Milk, skim (24 hours old)	1.0	None	None	None	None.
Milk, skim, plus $Ca(OH)_2$	1.0	Good	Good	Good	Good.
Gelatin	.1	do	do	do	Do.
Do	.01	Medium	None	None	None.
Glue	.1	Good	Good	Good	Good.
Saponin bark	1.0	do	do	do	Do.
Do	.1	Medium	None	Poor	Poor.
Tannin	.1	do	Poor	Medium	Do.
Gum arabic	1.0	None	None	None	None.
Pepsin	.1	Good	Medium	Medium	Medium
Flour, hard wheat	.1	do	Good	Good	Good.
Do	.01	Medium	Poor	Medium	Poor.
Agar agar	.1	do	do	Poor	Medium.
Do	.01	do	do	do	Poor.
Blood (water extract)	1.0	Good	Good	Good	Good.
Do	.1	Medium	Poor	Poor	Medium
Albumin	.1	Good	Good	Good	Good.
Do	.01	do	do	do	Do.
Red clover hay (extract)	2.0	do	do	do	Good.
Do	.2	Medium	Poor	Poor	None.
Vetch hay (extract)	2.0	Good	Good	Good	Good.
Alfalfa hay (extract)	2.0	do	do	do	Do.
Creosote oil	1.0	None	None	None	None.
Creosote, beechwood	1.0	do	do	do	Do.
Cresol	.1	Medium	do	do	Poor.
Evgenol	.5	do	do	Poor	None.
Phloroglucine	.1	Poor	do	None	Do.
Guaiacol	.1	do	do	do	Do.
Resorcin	.1	do	do	do	Do.
Oil of cottonseed	.1	Medium	do	Poor	Do.
Oil of cedar	.1	do	do	do	Do.
Oil of pears	.1	None	do	None	Do.

gravity of 0.869 at 20° C. was selected and observations made on the interfacial tension of many substances to the oil. The surface tension of the oil was 30.4 dynes per centimeter at 25°.

The apparatus devised by Fahrenwald (*2*) and used for these surface-tension determinations was used also for interfacial determinations. It is claimed that this is the only instrument made that will give fairly true static interfacial tension values. This is important in that when a spray issues from the nozzle of the spray gun in drops of various sizes it establishes, at least partially, static equilibrium by the time it comes into contact with and settles upon the plant surface. After contact, static equilibrium is established at the interface of spray solution to plant surface, quickly or slowly, depending upon the spreader solution used. Measurements were recorded about 3 minutes after the oil was introduced upon the surface of the spreader solution. Table III gives the results of the observations made at 25° C.

TABLE III.—*Interfacial tension between paraffin oil and miscellaneous substances in solution*

Material	Concentration	Interfacial tension	Material	Concentration	Interfacial tension
	Per cent	*Dynes per cm.*		*Per cent*	*Dynes per cm.*
Water		24.4	Casein (in 0.05 per cent $NaHCO_3$)	0.1	9.2
Sodium palmate	0.1	11.0	Do	.01	11.5
Do	.01	19.7	Milk, skim (on dry basis)	1.0	9.4
Do	.001	22.1	Do	.1	10.1
Sodium stearate	.1	8.2	Do	.01	11.9
Do	.01	19.2	Gelatin (purified)	1.0	10.8
Do	.001	22.1	Do	.1	14.6
Sodium oleate	.1	3.8	Do	.01	17.4
Do	.01	17.1	Glue (commercial)	1.0	12.2
Do	.001	20.8	Do	.1	15.3
Potassium oleate	.1	5.5	Do	.01	17.5
Do	.01	18.1	Saponin (bark extracted with water)	1.0	5.0
Do	.001	21.4	Do	.1	5.4
Soap, Castile	.1	6.1	Do	.03	9.3
Do	.01	16.6	Tannin	1.0	10.1
Soap, Ivory	.1	10.2	Do	.1	18.0
Do	.01	18.6	Do	.01	19.3
Soap, linseed oil	.1	7.1	Gum arabic	1.0	15.4
Do	.01	12.6	Do	.1	18.6
Mineral oil emulsion No. 1	4.0	2.5	Do	.01	20.2
Do	2.0	6.0	Creosote (meta)	.1	20.2
Do	1.0	10.1	Creosote (beechwood)	.1	20.8
Do	.1	18.1	Cresol	.1	17.1
Mineral oil emulsion No. 2	4.0	7.5	Resorcin	.1	21.0
Do	2.0	10.6	Guaiacol	.1	20.8
Do	.1	18.1	Pyrogallol	.1	21.1
Casein (in saturated lime water)	1.0	8.5	Phloroglucine	.1	20.2
Do	.1	8.6	Evgenol	.1	19.0
Do	.01	11.5	Oil of cloves	.1	16.5
Casein (in 1 per cent $Ca(HO)_2$ suspension)	1.0	6.7	Oil of Eucalyptus	.1	16.0
Do	.1	8.1	Oil of cedar	.1	14.4
Do	.01	11.2	Oil of cedar in 10 per cent alcohol	.1	19.9
Casein (in 0.05 per cent $NaHCO_3$)	1.0	8.3	Oil of pears (amyl acetate)	.1	21.3

Correlating the results in Table III with the actual spreading properties of the different substances as observed in Table II, it is to be noted that the interfacial tension values, where oil is substituted as the solid, are not proportional to the spreading properties of the various materials. If conditions favorable to spreading are expressed in equation form, then:

$$A_t > B_t + A_tB_t$$

in which A_t is the surface tension of the solid, or surface to be covered; B_t, the surface tension of the liquid or spreader solution; and A_tB_t, the interfacial tension between the liquid and solid. By substituting the values given in Tables I and III one finds that negative results are obtained for most of the substances tested. This indicates that there is not complete wetting or spreading of the different substances on the oil used. As an example, the surface tension of the oil (A_t) was 30.4 dynes-cm.; the surface tension of a 1.0 per cent solution of sodium palmitate (B_t) was found to be 31.2 dynes-cm.; the tension at the solute-oil interface (A_tB_t) was 11.4 dynes-cm. Substituting these values in the inequality one obtains -12.2. Although good spreading was observed on the solid or surface tested in Table II, the negative value, -12.2, indicates the opposite on the oil as a solid. Other oils, both mineral and vegetable, were also used, but similar results were obtained. The oils used, therefore, can not be substituted for waxy or other surfaces of the plant to

obtain values whereby the spreading qualities of a solute may be estimated by substituting the surface tension values in the inequality just given.

Although specific deduction can not be made from the surface and interfacial tension values regarding the spreading properties of various solutes, possibilities may be suggested, especially if the chemical composition is taken into consideration. Low values indicate in general surface adsorption and all substances tested and showing this phenomenon have certain spreading qualities. There are probably other influencing factors that affect spreading. Such factors as the solvent action of the spreaders on the surface to be covered, the rapidity with which static equilibrium is established, and the formation of plastic solids at the interface may benefit or reduce spreading.

Further examination of the observations reported in Table II discloses why it is difficult to generalize on the spreading qualities of any particular substance. It will be seen that some materials at a certain dilution spread well on apple leaves, and "medium" or "poor" on pears or prune leaves. Other variations are shown as "medium" spread on apple and prune leaves, while only "poor" spreading is obtained on pear leaves and apple fruit. It was also noticed that the age and exposure of the leaf to the sun's rays influenced the degree of spreading. For example, a solute at minimum concentration would spread nicely on a young apple leaf, while barely perceptible spreading would occur on an old leaf taken from the exposed side of the tree. In many cases, however, a higher concentration of the spreader gave better results. Ruth and Kelley [4] likewise observed that it was very difficult to wet certain areas of a tree, that differences in surfaces existed, and that the surfaces changed throughout the growing season. This change in surface, whether leaf or fruit, probably accounts for contradictory results obtained by different workers using the same kind of spreader. Under these circumstances it would be futile to conclude from laboratory tests that a substance would spread on all surfaces.

In a search for better and more practical spreaders many substances and combinations of two or more materials were studied. As negative results were obtained, many of them are not reported in the tables. The materials that aided best in producing a film over the surfaces tested may be divided into two classes, namely, the soaps and the substances containing water soluble or colloidal solutions of proteins. In general, the soaps required much higher concentration than the soluble proteins to produce equal spreading on most of the surfaces tested. Contrary to Moore's opinion that spreading of casein would not occur unless sufficient force was applied to penetrate the wax covering of the cabbage leaf, a good film was formed with most of the soluble protein materials, when sprayed to the drenching point. The force necessary depended upon the concentration of the protein, being greater for the lower concentrations and less at the higher concentrations. This indicates a flattening of the droplets by force until they cohere to form a continuous film instead of penetrating the waxy surface. The same holds true for the waxy covering of the apple.

[4] RUTH, W. A., and KELLEY, V. W. RECENT ADVANCES IN SPRAYING. Unpublished data 1922.

When it was observed that solutions containing soluble proteins spread well on most surfaces, the study of this class of material was extended to include many other similar substances. Among these, albumin, pepsin, hot water extracts of dried blood and of sage, dried skim milk powder and hot water extracts of such hays as clover, vetch, alfalfa, and dried grasses, spread well on all surfaces tested, even at low concentrations. This further substantiates the opinion that the soluble protein present is the active spreading agent. It is probable also that the active elements in the "Irish moss" used successfully by both Issleib (*5*) and Stearns (*9*) were soluble protein substances.

The use of spreaders for general orchard sprays has become more prevalent during the last few years. The development of the commercial casein-lime product has contributed mainly to this increase. Questions regarding the advisability of using spreaders, the kind to use, and the concentration that will give best results can be answered satisfactorily only when all conditions are taken into consideration. The cost is perhaps the most important point to consider. If the cost is low, spreaders may be used advantageously in most sprays, but if they are as high as present prices that prevail for the commercial casein-lime it should be used only for those sprays that unquestionably are improved by it and when beneficial results are obvious. It does not seem justifiable under average conditions to pay as much for the spreader as for the spray material itself, where comparative results indicate only a slight advantage in control. Under abnormal conditions and in parts of the country where severe epidemics of disease or insects prevail, their general use may be justified. The use of spreaders also in the last two cover sprays for apples and pears may be advisable in order to obviate the necessity of wiping the fruit and to allow a more even color development.

If the cost of a spreader is low enough to allow a satisfactory profit to the grower, then the more general use of spreaders is advisable.

Considering price, skim milk and skim-milk products have proved to be excellent substitutes for the commercial casein-lime spreaders. Laboratory tests and limited field experiments indicate that when skim milk is used at the rate if 2 to 3 quarts to 100 gallons it spreads well over many surfaces such as apple, pear, prune, peach, cherry, and cabbage leaves, and apple and pear fruits. The dried product used in equivalent amounts, as at the rate of solids in skim milk or 2.5 ounces to the quart, and the partially dried clabbered milk such as is used for stock feed, give equally good results. It is necessary to add a few ounces of hydrated lime or slaked quicklime to each quart of sweet skim milk and larger amounts to the commercial stock foods in order to neutralize the acidity and to bring the proteins into colloidal solution. Unless the lime is added spreading will not result. Skim milk, the dried or condensed skim milk, and the clabbered product are brought into solution easily, and in this respect they are preferable to the commercial casein-lime that must be worked carefully to a paste before putting into the spray tank in order to get best results.

THE EFFECT OF SPREADERS ON THE AMOUNT OF POISON THAT ADHERES

When a good spreader is used the whole surface is wetted and an even coating of the spray is obtained. As the film is formed the excess runs off and gives the appearance of excessive drip. In order to learn whether less poison adheres to the surface when a spreader is used, a series of laboratory tests were made. Apple and pear leaves were sprayed with lead arsenate at several dilutions with and without a spreader in the spray. Milk, commercial casein-lime, and laboratory preparations of casein and hydrated lime were used as spreaders. The leaves were sprayed with an atomizer and suspended at a 45° angle to dry and to allow the excess to drain off. The surface areas of the leaves were measured by means of a planimeter. The amount of arsenic that adhered to 100 square inches of leaf surface was then determined. The results obtained are given in Table IV.

TABLE IV.—*The amount of lead arsenate on 100 square inches of leaf surface*

Lead arsenate concentration	Spreader treatment	Apple	Pear
		Mgm.	*Mgm.*
2 to 100	None	7.2	4.9
2 to 100	Casein 0.05 per cent in saturated $Ca(OH)_2$ solution	6.8	6.2
2 to 100	Casein 0.05 per cent+$Ca(OH)_2$ 0.08 per cent	6.1	5.6
4 to 100	None	12.0	16.0
4 to 100	Casein 0.10 per cent in saturated $Ca(OH)_2$ solution	9.3	5.5
4 to 100	Casein 0.05 per cent+$Ca(OH)_2$ 0.08 per cent	10.0	8.5
4 to 100	Skim milk 1.4 per cent+$Ca(OH)_2$ 0.1 per cent	13.1	7.2
4 to 100	Whole milk 1.4 per cent+$Ca(OH)_2$ 0.05 per cent	12.0	11.0
4 to 100	None	11.8	16.4
4 to 100	Casein 0.01 per cent+$Ca(OH)_2$ 0.02 per cent	8.8	11.3
4 to 100	Casein 0.02 per cent+$Ca(OH)_2$ 0.04 per cent	15.5	10.4
4 to 100	Casein 0.05 per cent+$Ca(OH)_2$ 0.1 per cent	8.3	11.4
4 to 100	Commercial casein-lime 0.15 per cent	11.1	11.9
6 to 100	None	19.0	23.5
6 to 100	Casein 1 per cent in saturated $Ca(OH)_2$ solution	10.2	13.3
6 to 100	Casein 0.02+$Ca(OH)_2$ 0.04 per cent	21.2	12.6
6 to 100	Skim milk 2.0 per cent+$Ca(OH)_2$ 0.04 per cent	19.5	12.4
6 to 100	Skim milk 2.0 per cent+$Ca(OH)_2$ 0.08 per cent	17.0	11.0

It will be noted from the results given in Table IV that there is an appreciable variation which, perhaps, may be due to the age and size of leaves used and to the amount of lead arsenate that drained to the lower tip of the leaf. In general, however, it may be said that at all dilutions the apple leaves sprayed without a spreader show no more arsenic than where a spreader had been used. With the pear leaves, larger amounts of lead arsenate were found on the leaves sprayed without a spreader, except at the 2:100 dilution. However, the leaves sprayed without a spreader were covered in spots only, while where a spreader was used the coating of poison was an even film.

The amount of lead arsenate that adhered to the leaves was, for the most part, proportional to the concentration used. Approximately the same amount of lead arsenate was found on leaves where milk had been used in the spray as with the various forms of casein-lime.

In addition to the laboratory tests, samples of leaves sprayed under normal conditions in the orchard were collected and the amount of lead arsenate that adhered was determined. Several different

commercial brands of lead arsenate were used. The leaves were collected a few hours after spraying and the surface area measured as before. Table V gives the results obtained.

TABLE V.—*The amount of lead arsenate on 100 square inches of leaf surface sprayed in the orchard*

Lead arsenate	Concentration	Spreader used	Mgm. per 100 square inches
No. 1	2 to 100	None	26.4
Do	2 to 100	Casein 0.05 per cent	31.6
Do	2 to 100	Skim milk 0.60 per cent	36.2
Do	2 to 100	Casein 0.05 per cent	25.2
No. 2	2 to 100	None	24.3
Do	2 to 100	Casein 0.05 per cent	29.3
No. 3	4 to 100	None	45.2
Do	4 to 100	Casein 0.05 per cent	55.5

From the results in Table V it will be noted that much more lead arsenate adhered to the leaves sprayed under orchard conditions than when sprayed in the laboratory. This may be due to the carbon dioxide of the breath used to force the spray on the leaves and to the force used in applying the spray. The amount of lead arsenate that adhered was generally higher on the leaves where a spreader had been used than where lead arsenate only had been applied. Skim milk, apparently, was as good as the casein-lime spreaders. It spread well and formed a good even coating of the poison on the leaf surface.

Both the laboratory and orchard experiments indicate that skim milk or casein-lime spreader may be used advantageously if properly applied. The amount of spreader to use for best results is an important phase of the work and must be decided by field observations. Many variable factors that exist at the time of application should be taken into consideration and the amount of spreader used should be controlled accordingly. The pressure or force used, the kind of surface to be covered, the type of gun or nozzle used, and the climatic conditions, may influence the amount of spreader that will give best results. Too much spreader may cause excessive drip if sprayed to the drenching point. On the other hand, drenching with good force with a minimum amount of spreader is advisable. Most of these points should be definitely settled by field observations.

SUMMARY

No definite proportional relationship could be established between the surface tension values of spreader solutions and the observed spreading properties of the different substances. However, a solution having a low surface tension or a low interfacial tension to oil probably has spreading properties.

Of the materials tried, water-soluble protein substances gave best spreading at lowest concentration for the greatest number of surfaces tested. Skim milk, neutralized with hydrated lime, and certain other milk products appear to be the best material for practical purposes.

The concentration of a spreader solution that will give best results depends upon a number of variable factors, such as the type and age of the surface to be sprayed, the force used, and climatic conditions.

The amount of lead arsenate that adheres to the leaf surface is approximately the same when a spreader is added as when one is not.

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ASSOCIATIONS BETWEEN NUMBER OF KERNEL ROWS, PRODUCTIVENESS, AND DELETERIOUS CHARACTERS IN CORN [1]

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INTRODUCTION

Two different methods of selecting seed corn have been used for many years in the southern part of the United States. Under one of them, seed has been selected from plants bearing two or more ears; under the other, seed has been selected on the basis of the size of the ear. As a result, two classes of varieties, prolific and nonprolific, are now in common use. In general, the prolific varieties bear more than one ear on a plant, whereas the nonprolific varieties bear only one. The ears in the prolific varieties are smaller, and it would seem that the yields of the two might be equal, the differences in size and in number of ears offsetting each other. Experiments with comparable varieties have shown otherwise. Studies of the ears in prolific and nonprolific varieties showed that there were characteristic differences between them in the number of rows of kernels on the ear and in the size and angularity of the kernels.

The effect of differences in these characters upon yield has been investigated, using groups of ears within both prolific and nonprolific varieties and within a number of F_1 varietal crosses. The relation of the number of rows of kernels to smut resistance, to freedom from abnormalities, and to general vigor and productiveness also has been studied in self-fertilized lines. It is the object of this paper to present the data from these experiments and to discuss their application to corn improvement.

EXPERIMENTAL METHODS

The soil and climatic conditions where the experiments were made usually gave yields of 30 to 50 bushels of corn per acre with a good local variety. The different varieties or selections compared required practically the same time to mature, which was well within the seasonal limits.

From 20 to 50 ears were used to represent each variety or selection. Seed ears were selected in the studies on number of rows, etc., that differed in the character being studied but that were as nearly alike in other respects as the material permitted. All of the seed used was examined closely, and defective or irregular kernels were eliminated before planting.

Specially constructed hand planters were used with which an exact number of kernels could be planted at a given place and covered uniformly. The hills were spaced 3 feet apart in the row, center to center, by means of a metal spacing cable. The number of plants per acre was adjusted in accordance with soil productiveness by regulating the space between the rows.

[1] Received for publication Sept. 17, 1924; issued September, 1925.

The hill-checking method was used in all comparisons—that is, one test plant and one check plant were grown in each hill. These two plants were 8 to 10 inches apart in the rows, and always in the same order so that they could be identified. The seed for the test plants in any one row was of one of the varieties or selections being compared. The seed for the check plants was composited by taking a definite number of kernels from a definite number of ears and was the same for all of the rows in the experiment.

In general, two kernels were planted where one plant was desired, the extra seedlings being removed when they were from 4 to 8 inches high. This partly eliminated irregularity in stand. The effect of the remaining irregularity in stand, as well as the effect of soil variation, was avoided by harvesting only perfect hills. A perfect hill was defined as one containing a check plant and a test plant that could be identified definitely and that apparently had had equal growing conditions.

Each variety or selection in an experiment was grown in single rows replicated in different parts of the plat. The perfect hills in these rows were harvested, and the production of the test plants and of the check plants was determined for each row. The weight of ears from the test plants in all rows of one variety or strain in the experiment, divided by the weight of ears from the corresponding check plants, expressed as a percentage, constitutes a relative yield as here used. It is believed that the reliability of results obtained by the hill-checking method is proportional in general to the total number of perfect hills grown. Accordingly, the number of perfect hills on which the relative yields are based is given in the data.

In the varietal experiment in 1912 moisture determinations were made on the shelled grain, and these are given in the data, but no corrections were made in the yields. In most of the experiments the ears were uniformly dry at harvest, or, if not, corrections were made on the basis of data from drying samples.

VARIETAL EXPERIMENTS

EXPERIMENTS IN SOUTH CAROLINA IN 1912

The varietal experiments in South Carolina in 1912 were conducted on 10 farms in different parts of the State. Ten varieties, each of which had been grown previously on one of these farms for three or four years, were used throughout the experiments. One hill-checked row of each of the 10 varieties constituted a section which was replicated 10 times, each of the varieties being used as the check in one section.

The total yield of all the plants of a variety in the experiment was divided by the total yield of all the plants grown in the same perfect hills with it to obtain its relative yield. Computed in this way, the relative yields are percentages of the average yield of all the varieties in the experiment.

The experiment was destroyed by storm and flood on one farm. Two varieties were found to be somewhat earlier than the others, and their yields are not presented. This leaves the yields obtained on 9 farms from 8 varieties, 4 prolific and 4 nonprolific, as a basis for comparison. It was evident that these widely distributed farms repre-

sented different growing conditions, so that the combined data from them may be looked upon as representing the average reaction of these varieties to nine different cropping seasons.

A summary of the data from these experiments is shown in Table I, those from the prolific and nonprolific varieties being grouped separately. The prolific varieties averaged more than one ear per plant, and the nonprolific averaged less than one ear. It is evident from the data that the nonprolific varieties must have had a considerable proportion of barren plants. The average relative yield of the prolific varieties was 9 per cent greater than that of the nonprolific, notwithstanding the fact that the ears contained less moisture [2] at the time the weights were taken. The most prolific variety in the experiment also was the most productive, and all of the prolific varieties were more productive than any one of the nonprolific except variety No. 5.

TABLE I.—*Relative percentage yields of prolific and nonprolific varieties on nine farms in different parts of South Carolina in 1912*

Prolific varieties					Nonprolific varieties				
Variety No.	Perfect hills	Relative yield	Moisture	Ears per plant	Variety No.	Perfect hills	Relative yield	Moisture	Ears per plant
		Per cent	*Per cent*				*Per cent*	*Per cent*	
7	5,933	119	16.20	1.45	5	5,963	115	17.65	0.95
4	5,714	113	16.29	1.29	2	5,528	100	17.97	.92
3	5,938	109	16.04	1.12	1	5,818	93	17.03	.97
8	5,941	106	15.81	1.28	10	6,052	88	17.70	.85
Total..	23,526	[a]112	----------	[a]1.28	------	23,361	[a]103	----------	[a].92

[a] Computed directly from the basic data.

EXPERIMENTS IN GEORGIA IN 1915

Varietal experiments were conducted on each of four farms near Thomasville, Ga., in 1915. The growing conditions on each farm were different, and the results may be considered as the average reaction from four cropping seasons.

In planning these experiments it was the intention to include all of the varieties with outstanding characteristics that are commonly grown under climatic conditions similar to those under which the experiments were conducted. Twelve of the thirteen varieties included were studied carefully on the farms from which they were obtained in the fall of 1914, and the seed ears for planting were characteristic of the varieties. A summary of the data from these experiments is shown in Table II, the prolific and nonprolific varieties being grouped separately.

As a class, the prolific varieties had larger yields, more ears per plant, smaller percentages of barren plants, smaller ears per productive plant, and fewer rows of kernels per ear, than did the nonprolific varieties.

[2] The moisture determinations were made from samples of shelled grain taken from each plat. They do not take into consideration the moisture in the cobs. As the yields are based on the weight of ear corn, no attempt was made to correct for differences in moisture.

TABLE II.—*Relative percentage yields of prolific and nonprolific varieties on four farms near Thomasville, Ga., in 1915*

Variety	Perfect hills	Relative yield	Ears per plant	Length of total ears per bearing plant (A)	Average diameter of ears (B)	Yield index ($A \times B_2$)	Barren plants	Kernel rows per ear
Prolific varieties:		*Per cent*		*Inches*	*Inches*		*Per cent*	
Garrick	495	146	1.32	7.9	1.9	28.5	4.8	11.61
Wannamaker	476	142	1.20	7.9	1.9	28.5	5.5	12.41
Weekley	482	130	1.25	7.2	1.8	23.3	6.2	12.84
Whaley	408	126	1.29	7.4	1.8	24.0	6.1	12.64
Whatley	467	124	1.59	6.3	1.8	20.4	5.1	13.38
Round	472	103	1.34	7.3	1.6	18.7	8.9	11.44
Littlecob	308	86	1.29	7.8	1.5	17.6	7.1	10.18
Total or average	3,108	[a]124	1.33	7.4	1.8	23.0	[a]6.2	12.07
Nonprolific varieties:								
Loveless	462	110	.86	8.4	1.9	30.3	9.7	13.62
Watson	448	107	.98	7.8	1.9	28.2	14.1	13.60
Stone	327	98	.88	8.0	1.9	28.9	14.7	13.96
Cochran	464	87	.91	7.5	1.8	24.3	16.8	13.68
Gwaltney	395	87	.90	7.2	2.0	28.8	14.7	12.64
Laguna	390	60	.91	7.3	2.1	32.2	15.5	14.69
Total or average	2,486	[a]93	.91	7.7	1.9	28.8	[a]14.1	13.70

[a] Computed directly from basic data.

The data in column 7 of Table II were obtained by multiplying the average length of total ears per bearing plant by the square of the average diameter of the ears. This gives an index of the volume of ear produced per bearing plant and consequently an index to the yield of the bearing plants. On the basis of the yield index the prolific varieties made their higher yield with a production per bearing plant that actually was less than that of the nonprolific varieties.

Assuming that selection aims to produce the largest individual plant yields, the breeders of the nonprolific varieties had evidently done more effective work than the breeders of the prolific varieties, if only the bearing plants are considered. For some reason, however, the returns were smaller. This was due to the fact that the nonprolific varieties had a disproportionate number of plants without grain. Each of the nonprolific varieties had a larger percentage of barren plants than any one of the prolific varieties, the average percentage of barren plants being 14.1 per cent for the nonprolific group and 6.2 per cent for the prolific group.

COMPARISONS WITHIN VARIETIES

In general, the ears in the nonprolific varieties in the preceding experiments had more kernel rows and more angular, tighter-fitting kernels than the ears in the prolific varieties. In order to study the relation of such differences to yield, a series of experiments was begun in 1914. In all of these experiments, groups of ears were selected that differed in one or more characters of the ears or kernels. The relative productiveness of these groups was then determined.

Many experiments have been conducted on the relation between yield and the different characters of seed ears. The results of these were summarized by Richey [3] in 1922 as follows:

[3] RICHEY, F. D. THE EXPERIMENTAL BASIS FOR THE PRESENT STATUS OF CORN BREEDING. Jour. Amer. Soc. Agron. 14: 4. 1922.

Inasmuch as these data are based on comparisons between ears all of which were suitable for seed, the preponderance of evidence in certain cases seems convincing in spite of the fact that the determining differences in yield are small. There is every indication that selection on the basis of production (weight of ear in this case) is of value. Likewise it is indicated that it is preferable to obtain production by adding to the length rather than to the circumference of the ears, and that smoother, fewer-rowed ears with a lower shelling percentage than the standard show type are inclined to be the better yielders.

Kiesselbach [4] stated in substance that in a six-year comparison the long, slender, smooth ears surpassed all other kinds, including the long, large, rough ears; the short, large, rough ears; the short, slender, smooth ears; and unclassified seed of Nebraska White Prize, the variety from which the selections were made. He does not state but shows in an illustration that the most productive type had markedly fewer rows than the ordinary Nebraska White Prize. He also shows in the same bulletin that hybrids producing smooth, few-rowed ears yielded more than hybrids producing ears with a larger number of rows and rougher kernels.

Several experiments were conducted at different points in South Carolina and Florida. The performances of groups of ears differing in the number of rows of kernels were compared at Lykesland, S. C., in 1914, 1916, and 1917; at Brooksville, Fla., in 1917; and at Darlington, S. C., in 1915 and 1916. In the experiments at Lykesland the ears with different numbers of rows were classified further on the basis of angularity of kernel in 1914 and on the basis of kernel size in 1916. Additional studies of the relation of angularity of kernel to yield were made within a variety at Lykesland in 1914 and 1915, and at Darlington in 1915, and within two series of varietal crosses at Darlington in 1914. Summaries of the data from these experiments are given in Tables III, IV, V, VI, and VII.

NUMBER OF KERNEL ROWS AND YIELD

Data on the relation of number of kernel rows to yield in the Roger and Williamson varieties are shown in Table III. These are typical nonprolific varieties, the ears being rough, with comparatively many kernel rows and tightly spaced angular kernels. Three groups of ears in each variety were compared in 1915, and six groups in the Roger variety were compared in 1916.

TABLE III.—*Data on the relation of the number of kernel rows to yield in the Roger and Williamson varieties at Darlington, S. C., in 1915 and 1916*

Points cons dered	1915						1916					
	Williamson			Roger			Roger					
Number of rows on seed ears	12 and 14	16 and 18	20	12	14 and 16	18	10	12	14	16	18	20
Number of perfect hills grown	602	506	530	462	414	453	730	751	763	778	727	700
Relative yields	108	107	97	100	94	88	98	100	105	96	94	95

The yields were inversely proportional to the number of kernel rows in both varieties in 1915. The highest yield in 1916 was from

[4] KIESSELBACH, T. A. CORN INVESTIGATIONS Nebr. Agr. Exp. Sta. Research Bul. 20, 151 p. 1922.

the ears with 14 rows, the modal number in the Roger variety. Each of the three few-rowed groups (10, 12, and 14 rows), however, yielded more than any one of the three many-rowed groups.

The data show a consistent relation between the number of rows on the seed ears and the yield produced from them, the ears with the lower number of rows yielding more as a class than those with the higher number of rows.

The Garrick variety was included in both the South Carolina and Georgia varietal experiments and was highest yielding in each. From the standpoint of selection, this variety is the most perfect product of the prolific ideal that was studied. It was learned by interview that Mr. Garrick had grown this corn at Weston, S. C., for about 30 years. His ideal had been the production of two or more ears per plant, and he had given no special attention to the character of the ears and kernels. Several thousand ears of this variety were studied on the home farm. The ears were large for the prolific class of corn, the kernels were intermediate between angular and rounded, and the characteristic numbers of rows of kernels on an ear were 10 and 12. The indentation was distinct and smooth to slightly rough.

Data on the relation of number of rows of kernels to yield in the Garrick variety are shown in Table IV. As different lots of ears were used in each experiment, eight distinct comparisons are shown. Ears with the fewest rows of kernels gave the highest yields in six comparisons, and ears with the most rows gave the lowest yields in seven comparisons. Ears with 12 rows of kernels yielded more than those with more than 12 rows, in all of the eight comparisons.

TABLE IV.—*Data on the relation of number of kernel rows, angularity of kernels, and weight of kernels in the parent ears to yield in the Garrick variety grown at different places and in different seasons*

Place and date of experiment, and character of kernel classes compared in 1914 and 1916	Relative yields from ears with stated number of kernel rows (per cent)						Perfect hills grown from ears with stated number of kernel rows						Weight of kernels (grams) per 1,000 from ears with the stated number of kernel rows			
	8	10	12	14	16	Average [a]	8	10	12	14	16	Total	10	12	14	Av.
Lykesland, S. C., 1914:																
Kernels angular		91	84	83		87		317	291	295		903				
Kernels intermediate		99	101	96		99		319	295	292		906				
Kernels rounded		89	93	84		88		264	300	315		879				
Total or average [a]		94	93	87				900	886	902						
Lykesland, S. C., 1916:																
Kernels large		119	100	89		102		606	619	657		1,882	406	418	374	419
Kernels midsized		101	92	86		93		644	685	595		1,924	387	378	346	370
Kernels small		94	93	86		91		715	602	671		1,988	358	334	324	338
Total or average [a]		104	95	87				1,965	1,906	1,923			403	377	348	
Lykesland, S. C., 1917	112		109		100		1,085		579		572					
Brooksville, Fla., 1917	104	101	100	94	93		650	630	1,220	1,144	509					

[a] Computed directly from the basic data.

Both in the prolific and the nonprolific varieties that were studied there was a strong general tendency for the ears with fewer rows of kernels to yield more than those with larger numbers of rows.

ANGULARITY OF KERNEL AND YIELD

One characteristic difference between the prolific and nonprolific varieties was in the shape of the kernels. The nonprolific varieties tended to have more angular kernels that are crowded more closely on the ear. This angularity probably is due, in part at least, to the greater lateral pressure during their development.

Data on the relation of angularity of kernel to yield in one experiment with the Garrick variety, in which the ears with rounded kernels yielded 9 per cent more than those with angular kernels, are shown in Table V.

TABLE V.—*Data on the relation of angularity of kernels on the seed ears to the number of kernel rows and to yield in the Garrick variety at Lykesland, S. C., in 1914*

Angularity of kernels on seed ears	Kernel rows on seed ears (average)	Perfect hills	Relative yields
			Per cent
Angular	13.2	1,542	93
Rounded *	10.8	1,735	102

* This class included kernels of both the intermediate and rounded classes of other experiments.

In 1913, one row of plants of each of 15 varieties was grown in a field of the Roger variety and detasseled before pollen was shed. A similar series was grown in a field of Garrick. The ears of the resulting crosses were grouped on the basis of angularity of kernel and these groups were compared, with the results shown in Table VI.

TABLE VI.—*Data on the relation of angularity of the kernels on the parent ears to yield in two series of varietal crosses at Darlington, S. C., in 1914*

Shape of kernels	15 varieties crossed by Roger			15 varieties crossed by Garrick		
	Rows on parent ears (average)	Perfect hills	Relative yields	Rows on parent ears (average)	Perfect hills	Relative yields
			Per cent			*Per cent*
Angular	16.1	1,882	105	14.8	1,919	119
Intermediate	12.9	1,848	110	12.9	1,934	123
Rounded	13.7	1,885	108	13.7	1,898	125

The number of plants of each female parent variety was so limited that the rounded-kernel class was poorly represented in some crosses.

The ears with angular kernels produced the lowest yields in both series of crosses. The ears with kernels of intermediate angularity produced the highest yields in the Roger crosses, whereas the ears with kernels of intermediate angularity produced more than the ears with angular and less than those with rounded kernels in the Garrick crosses.

In the preceding experiments the ears with angular kernels had more rows of kernels than those with rounded kernels. To avoid any possible effect of the number of kernel rows on yield, the classi-

fication for angularity also was made within groups of ears of the Garrick variety having the same number of rows of kernels. Data from these experiments are given in Tables IV and VII.

As the data in Table IV are for different lots of ears and those in Table VII are for the same lots of ears grown in different places, four distinct comparisons are provided in the two tables. The ears with kernels of intermediate angularity produced more than either of the extreme classes in all four comparisons. In two of the comparisons the ears with rounded kernels produced significantly more than those with angular kernels and in the other two comparisons the differences were negligible.

SIZE OF KERNEL AND YIELD

In connection with the experiments at Lykesland in 1916, the ears which had been classified for number of rows of kernels were grouped further into those with large, midsized, and small kernels. The data from this experiment are shown in Table IV.

TABLE VII.—*Data on the relation of weight and angularity of kernels on the parent ears to the yield of 12-rowed ears of the Garrick variety grown at Darlington and Lykesland, S. C., in 1915*

Shape of kernels	Weight per 1,000 kernels	Grown at Darlington, S. C.		Grown at Lykesland, S. C.	
		Perfect hills	Relative yields	Perfect hills	Relative yields
	Gm.		*Per cent*		*Per cent*
Angular	382	958	88	765	88
Midangular	399	905	99	798	102
Midrounded	397	3,852	102	3,330	102
Rounded	379	3,778	96	3,332	97

The yields tended to be directly proportional to the size of the kernels, and the yield of the ears having both the fewest rows and the largest kernels was very distinctly the highest. It will be noted in Table VII that the ears with midangular and midrounded kernels had heavier kernels than those of the extreme classes. The larger yields of these intermediate groups, therefore, may have been a result of their having heavier kernels, although this is not certain.

INTERRELATION OF NUMBER OF KERNEL ROWS, ANGULARITY OF KERNEL, AND SIZE OF KERNEL

It has been shown in connection with some of the preceding experiments that angularity of kernel is related to weight of kernel and that both of these characteristics are related to the number of rows of kernels on an ear. These interrelations probably are universal within a variety, and it is not possible to separate the effect of each individual factor in these experiments. Fortunately, however, this is not necessary from the present point of view, as it is necessary to know only that ears chosen within a variety to represent similar combinations of these characters react in a similar manner. Both in the varietal experiments and in the comparisons within varieties, the groups of ears with fewer rows of kernels and with less angular kernels have produced higher yields than those with more rows and more angular kernels

EXPERIMENTS WITH SELF-FERTILIZED LINES

Systematic selection within self-fertilized lines in several varieties has given comparatively quick results in the isolation of strains with distinct characteristics. In addition, it has afforded a view of some indirect effects of selection that could not be seen in the studies with open-fertilized material. Some of the results are given in the following pages.

EFFECT OF SELECTION UPON THE NUMBER OF ROWS OF KERNELS

Many plants of the Garrick variety were selfed in 1916 and good seed ears were selected from these to represent six classes with different numbers of kernel rows on an ear. These ranged from 8 to 18 rows, inclusive. These classes were maintained in the succeeding years by continuous selection. The effect of the selection during the first five years upon the percentage of progeny ears having the same number of kernel rows as the parent ear is shown in Table VIII.

TABLE VIII.—*Data on the effect of selecting continuously within selfed lines for different numbers of kernel rows, upon the percentages of the progeny conforming to the parental number of kernel rows*

Kind of data	Kernel rows on parent ears	Years in which the progeny were grown				
		1917	1918	1919	1920	1921
Number of parent ears	8	18	8	15	52	49
	10	24	8	19	64	69
	12	24	5	11	53	76
	14	21	4	10	52	76
	16	18	4	8	37	40
	18	3	4	2	15	16
Number of progeny ears classified	8	3,205	1,076	2,653	2,563	2,499
	10	4,790	1,050	3,486	3,513	4,642
	12	4,903	597	1,750	2,352	3,804
	14	3,095	498	1,198	2,147	3,432
	16	3,410	441	835	1,332	1,516
	18	572	477	197	530	612
		Per cent	*Per cent*	*Per cent*	*Per cent*	*Per cent*
Percentages of progeny ears having the same number of kernel rows as the parent ear.	8	21.2	25.4	47.7	68.6	85.6
	10	45.9	44.2	55.9	63.1	66.5
	12	38.7	38.6	44.9	46.8	52.9
	14	19.1	30.6	33.6	36.3	50.3
	16	11.8	13.0	26.1	32.9	43.9
	18	8.6	4.2	19.7	22.5	43.8
Highest percentage of progeny ears in an individual earrow having the same number of kernel rows as the parent ear.	8	35.9	32.3	86.6	100.0	100.0
	10	66.2	56.8	75.7	93.3	95.0
	12	51.0	49.0	66.9	81.3	77.0
	14	46.2	35.0	45.8	64.3	88.0
	16	26.3	20.6	33.9	59.3	63.0
	18	25.8	11.3	30.9	46.2	60.0

With the exception of the 10, 12, and 18 rowed classes in 1918, there was an annual increase throughout the five years in the degree to which the progeny ears in each class conformed to the parental character. The rate of increase, however, was not the same for each class. In the parent variety the mode for number of kernel rows was 12. During the first three selfed generations the 10-rowed class led in percentage of conformity to the parental number and the 8-rowed group took and maintained the lead after that. In the data for 1920 and 1921 the percentage of conformity to the parental number tended to be inversely proportional to the number of rows on the ears.

INDIRECT EFFECT OF SELECTION ON INDENTATION AND ANGULARITY OF KERNELS

After the Garrick lines had been selfed and selected for different numbers of kernel rows for five years they were examined to determine whether there had been any indirect effect upon the indentation and shape of the kernels. The results of this examination are shown in Table IX, the classifications for indentation and angularity being based upon the characteristic tendency of the ears in the individual ear rows. These studies were made at the Arlington Experiment Farm, Rosslyn, Va., and in cooperation with the Pee Dee Substation of the South Carolina Agricultural Experiment Station, Florence, S. C.

TABLE IX.—*Data on the effect of selecting for different numbers of kernel rows upon the indentation and angularity of kernels of self-fertilized lines of Garrick grown at Florence, S. C.,[a] and Rosslyn, Va., in 1921*

Kernel rows on parent ears	Ear rows grown	Percentages of ear rows producing ears with mode for kernel indentation stated					Percentages of ear rows producing ears with mode for angularity of kernels stated		
		None	Trace on only a few kernels	Shallow and smooth	Mid-depth and wrinkled	Deep and rough	Rounded	Intermediate	Angular
		Per cent	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*
8	46	26.1	6.5	45.7	19.6	2.2	69.6	28.3	2.1
10	59	16.9	6.8	52.5	13.6	10.2	59.3	32.2	8.5
12	59	18.6	8.5	42.4	16.9	13.6	55.9	33.9	10.2
14	65	7.7	1.5	50.8	20.0	20.0	46.2	40.0	13.8
16	52	5.8	3.8	38.5	26.9	25.0	42.3	28.8	28.8
18	34	8.8	0.0	35.3	20.6	35.3	44.1	20.6	35.3

[a] The data at Florence, S. C., were obtained in cooperation with the Pee Dee Substation of the South Carolina Agricultural Experiment Station.

The classification for indentation was more or less difficult, but it is believed that it was fairly accurate in the extreme classes. The largest percentages of ear rows with no indentation were from the parents with 8, 10, and 12 kernel rows, and the largest percentages in the class with deep and rough indentation were from parents with 14, 16, and 18 kernel rows. It is evident from this that selection for low numbers of rows on the ears tended to result in a different indentation from that obtained by selecting for higher numbers of kernel rows. Similarly, rounded kernels naturally followed selection for low numbers of kernel rows, and angular kernels followed selection for higher numbers of rows.

RELATION OF SIZE OF EARS TO NUMBER OF ROWS OF KERNELS

All of the ears produced in the Garrick selfed lines in 1921 were measured as to length and diameter. The total length of all the ears from each ear row was divided by the number of bearing plants in that row to obtain the total length of ear per plant. A yield index then was computed by multiplying this value by the square of the average diameter of the ears in the row. These data, and the average number of ears per plant produced by parent ears with different numbers of kernel rows, are shown in Table X.

TABLE X.—*Effect of selecting for numbers of rows of kernels upon number of ears per bearing plant, and length and diameter of ears, in selfed lines of Garrick grown at Florence, S. C.,[a] and Rosslyn, Va., in 1921*

Kernel rows on parent ears	Total progeny studied		Ears per plant	Average length of total ears per bearing plant (*A*)	Average diameter of ears (*B*)	Yield index ($A \times B^2$)
	Number of plants	Number of ears				
				Inches	*Inches*	*Inches*
8	1,091	2,366	2.17	13.21	1.48	28.94
10	1,742	3,493	2.01	12.86	1.58	32.10
12	1,587	2,944	1.86	12.67	1.68	35.76
14	1,757	3,209	1.83	12.13	1.75	37.15
16	1,348	2,091	1.55	9.92	1.81	32.50
18	867	1,335	1.54	9.76	1.85	33.40

[a] The data at Florence, S. C., were obtained in cooperation with the Pee Dee Substation of the South Carolina Agricultural Experiment Station.

The number of ears per plant tended to be inversely proportional to the number of rows on the parent ear and directly proportional to the length of ear per plant. The length of total ears per bearing plant tended to be inversely proportional, and the average diameter of ear tended to be directly proportional to the number of rows on the parent ears. The yield indexes were largest in the two middle classes for number of rows of kernels. The classes with 16 and 18 rows have larger yield indexes than the classes with only 8 or 10 rows. The yield indexes are based only on bearing plants and the differences are small. Inasmuch as the percentage of barren plants (Table XI) was materially larger in the classes with more kernel rows, the acre yield of the fewer-rowed ears evidently was larger. This agrees, in general, with the data previously presented from open-fertilized corn.

RELATION OF DELETERIOUS CHARACTERS TO NUMBER OF ROWS OF KERNELS

Many abnormalities occur among corn plants in lines that have been self-fertilized in successive generations. Some of these are eliminated by selection and others become more or less fixed and characteristic of individual hereditary lines. All of these abnormal characters have a more or less deleterious effect upon yield.

After five generations of selfing and selection for different numbers of kernel rows, data were obtained on the proportion of plants in the different Garrick lines having certain deleterious characters. The characters considered were: Plaited and erect leaves, entangled leaves, chlorophyl blotch, dead blotch, red and yellow flame, and barrenness. The dead blotch and the red and yellow flame characters both result in the premature death of the plants having them.

The data were obtained on a total of 4,013 plants in 158 ear rows grown in cooperation with the Pee Dee Substation of the South Carolina Agricultural Experiment Station, at Florence, S. C., and on 3,167 plants in 105 ear rows grown at the Arlington Experiment Farm, Rosslyn, Va. Notes were taken on all of the deleterious characters in the experiment in South Carolina, but in the experiment in Virginia barrenness and red and yellow flame were the only ones noted. A summary of the data from these experiments, in so far as they relate to the present problem, is shown in Table XI, the data being grouped according to the number of rows on the parent ears.

In both experiments the lines from parent ears with 8, 10, and 12 rows had smaller percentages of plants affected than the lines from parent ears with 16, 18, and 20 rows. This was true for each of the deleterious characters considered, although the differences in plants affected with dead blotch and chlorophyl blotch were small.

TABLE XI.—*Data on the relation of number of kernel rows on parent ears to percentages of plants having deleterious characters in selfed lines of Garrick grown at Rosslyn, Va., and Florence, S. C.,[a] in 1921*

Deleterious characters	Percentage of plants affected[b]		Deleterious characters	Percentage of plants affected[b]	
	Parent ears having 8, 10, and 12 rows	Parent ears having 16, 18, and 20 rows		Parent ears having 8, 10, and 12 rows	Parent ears having 16, 18, and 20 rows
Florence, S. C.:	*Per cent*	*Per cent*	Florence, S. C.—Continued.	*Per cent*	*Per cent*
Plaited and erect leaves	39.99	54.46	Barrenness	1.61	6.77
Entangled leaves	8.03	15.68	Rosslyn, Va.:		
Chlorophyl blotch	13.17	13.37	Red and yellow flame	1.89	7.40
Dead blotch	29.45	32.34	Barrenness	1.94	8.70
Red and yellow flame	.96	16.66			

[a] The data at Florence, S. C., were obtained in cooperation with the Pee Dee Substation of the South Carolina Agricultural Experiment Station.

[b] The total numbers of plants studied were 4,013 in South Carolina and 3,167 in Virginia.

RELATION OF SMUT RESISTANCE TO NUMBER OF ROWS OF KERNELS

The selfed lines in the Garrick variety were studied in 1922[5] to learn whether there was any evidence of resistance to smut. The studies were made at the Arlington Experiment Farm, Rosslyn, Va., on reclaimed land along the Potomac River where smut infection normally is high. The 138 ear rows that were studied represented 13 of the original selfed lines started in 1917 which had survived six generations of selfing and selection. Some of the ear rows in 5 of the 13 original lines or families were entirely free from smut. Three of these five families had been selected during the first five generations of selfing for 12 or less rows on the ear. The percentages of ear rows free from smut in these families were 33.3, 36.4, and 37.5. Two of the five families had been selected for 14 or more rows during the same period. The percentages of ear rows free from smut in these families were 5 and 25. The average percentage of smut-free ear rows in all of the lines from few-rowed parent ears was 12.2, and in all of the lines from many-rowed parent ears was 4.7.

Studies of smut resistance were continued with the Garrick lines in 1923 and additional investigations were begun with Cuban Yellow, a flint variety from southern Florida, and with Boone County White. Inbred lines of Cuban Yellow that had been selfed for four generations and lines of Boone County White that had been selfed three generations were used. In the selection of the lines in these varieties no attention had been given to the number of kernel rows on the ear. The experiments were conducted at the Arlington Experiment Farm, Rosslyn, Va.

[5] Notes on infection by smut and the artificial inoculations (referred to later) were made by W. H. Tisdale, Pathologist in Charge of Smut Investigations, Office of Cereal Investigations, Bureau of Plant Industry, United States Department of Agriculture, and his associates

In order to provide more opportunity for infection, stable manure with which large quantities of smut spores had been mixed was spread on the experimental plat in the spring. Later, a water suspension of conidia of the smut fungus from pure culture was applied to the growing points of the plants after they had been wounded with a sharp wire.

A summary of the data on freedom from smut in the Garrick lines in 1923 is given in Table XII, classified according to the number of kernel rows on the parent ears. Data on freedom from smut in the parent ear rows in 1922 also are given. All of the few-rowed parent ears used in this experiment were from ear rows that were entirely free from smut in 1922, as was one of the many-rowed parent ears. The other seven parent ears of the many-rowed class were from the most nearly smut-free ear rows of this class from which selfed ears were obtained in 1922. Inasmuch as approximately equal numbers of hand pollinations were attempted in the rows of both classes in 1922, this failure to obtain selfed ears from the many-rowed class furnishes an interesting side light on the greater vigor of the plants from the few-rowed parent ears.

TABLE XII.—*Data on the relation of number of kernel rows on the parent ears to freedom from smut in selfed lines of Garrick grown at Arlington Experiment Farm, Rosslyn, Va., in 1922 and 1923*

Family No.	Parent ears with 8 to 12 kernel rows						Family No.	Parent ears with 14 to 20 kernel rows					
	Parent rows, 1922			Progeny rows, 1923				Parent rows, 1922			Progeny rows, 1923		
	Ear row No.	Total plants	Smut-free plants	Ear row No.	Total plants	Smut-free plants		Ear row No.	Total plants	Smut-free plants	Ear row No.	Total plants	Smut-free plants
			P. ct.			*P. ct.*				*P. ct.*			*P. ct.*
49	76	20	100.0	2	89	49.4	86	45	20	95.0	20	117	29.9
				4	85	51.8		49	22	100.0	22	77	64.9
				5	103	79.6		60	26	88.5	23	84	31.0
	78	19	100.0	7	101	75.2							
				8	102	80.4							
54	26	17	100.0	10	107	77.6	99	51	18	94.5	25	132	62.1
				11	114	84.2					26	113	61.9
				13	97	93.8					29	108	61.1
	30	20	100.0	14	98	81.6		256	38	60.5	30	108	29.6
				16	113	85.0					31	115	17.4
				17	123	90.2							
Total ear rows		76	100.0		1,132	*78.2	Total ear rows		124	*83.9		854	*44.6

* Computed directly from the basic data

Only 2 of the 11 rows from ears with from 8 to 12 rows of kernels had lower percentages of smut-free plants than the most nearly smut-free rows from ears with 14 to 20 rows of kernels in 1923, and the rows from ears with fewer kernel rows averaged 33.6 per cent more smut-free plants than the rows from ears with more kernel rows.

The percentages of smut-free plants were lower in 1923 than in the parent-ear rows in 1922. This may have been due to a difference in the seasons, to the artificial inoculation of soil and plants in 1923, or to the fact that the data for 1922 are based only on ear rows as selected having the fewest smutted plants. In any event,

the difference in freedom from smut in the parent-ear rows of the two classes in 1922 was only 16.1 per cent, whereas the difference in 1923 was 33.6 per cent.

The data on freedom from smut in the experiment with the selfed lines of Cuban Yellow and Boone County White varieties are given in Table XIII, arranged according to the number of rows of kernels on the parent ears.

TABLE XIII.—*Percentages of smut-free plants in selfed lines of Cuban Yellow and Boone County White from parent ears with different numbers of rows of kernels, at Arlington Experiment Farm, Rosslyn, Va., 1923*

Variety	Rows from parent ears											
	12-kernel rows		14-kernel rows		16-kernel rows		18-kernel rows		20-kernel rows		22-kernel rows	
	Total plants	Smut-free plants	Total plants	Smut-free plants	Total plants	Smut-free plants	Total plants	Smut-free plants	Total plants	Smut-free plants	Total plants	Smut-free plants
		P. ct.		*P. ct.*		*P. ct.*		*P. ct.*		*P. ct.*		*P. ct.*
Cuban Yellow	97	73.2	88	51.1	110	31.8	119	47.9				
	106	78.3	100	65.0	106	67.0						
	108	60.2	107	58.9								
	92	78.3	112	58.9								
	110	63.6	87	71.3								
	111	59.5	109	55.0								
	118	81.4	108	69.4								
	111	40.5	114	52.6								
	106	50.0										
	106	54.7										
	109	77.1										
Total or average	1,174	*65.0	825	*60.1	216	*49.1	119	*47.9				
Boone County White	105	37.1	103	90.3	104	31.7	100	38.0	72	72.2	100	54.0
			99	47.5	106	83.0	86	69.8	97	78.4		
			111	91.9	89	47.2	82	72.0				
			95	48.4	91	84.6						
			69	73.9	98	69.4						
			97	83.5	94	51.1						
			105	73.3	99	86.9						
			59	44.1	93	46.2						
			83	67.5	107	89.7						
			65	49.2	112	43.8						
Total or average	105	*37.1	886	*69.0	993	*63.4	268	*58.6	169	*75.7	100	*54.0

* Computed directly from basic data.

In the Cuban Yellow variety, the ear rows having the largest percentages of smut-free plants were from ears with 12 rows of kernels, and the average percentage of smut-free plants decreased as the number of kernel rows increased.

In Boone County White, the ear rows with the largest percentages of smut-free plants were from ears with 14 rows of kernels. The one ear with 12 rows of kernels produced plants of which only 37.1 per cent was free from smut, and the two ears with 20 rows produced plants of which 75.7 per cent was free from smut. Omitting these classes, there was a consistent negative relation between the percentages of plants free from smut and the number of rows of kernels on the parent ears.

There were but few parent ears with numbers of rows other than those in the two principal classes in the lines of the Cuban Yellow

and the Boone County White varieties. Moreover, these lines had been selfed only for four and three generations, respectively. When the data are considered as a whole, however, they are in agreement with those obtained with the Garrick variety in indicating that lines characterized by relatively low numbers of rows on the ears are more resistant to smut than those characterized by larger numbers of kernel rows.

RELATION OF VIGOR TO NUMBER OF ROWS OF KERNELS

Reference already has been made to the selection of selfed lines of Cuban Yellow and Boone County White and to the fact that no attention was paid to the number of rows of kernels on the seed ears. These lines were selected on the basis of their general vigor, freedom from abnormality, and productiveness.

Most of the ears of the Cuban Yellow had 14 or 16 rows of kernels when selfing was begun. After four generations of selection for vigor, without reference to the number of rows of kernels, nearly all of the breeding ears had 12 or 14 kernel rows, the 12-rowed class being the largest (Table XIII). Most of the ears in the Boone County White variety had 16 or 18 rows of kernels when selfing was begun. After three generations of selection for vigor, without reference to the number of rows of kernels, 20 of the 27 breeding ears had 14 or 16 rows. Thus, selection for vigor and productiveness in the selfed lines of both of these varieties has decreased the characteristic number of rows of kernels on the ear.

The seventh selfed generation of the Garrick lines was grown at Baton Rouge, La., in 1923, in cooperation with the Louisiana Agricultural Experiment Station. Thirteen of the 108 original selfed lines of 1917 were represented in this planting and each of these lines or families was represented by from 3 to 13 ear rows.

The seed ears representing a family in 1923 varied somewhat in regard to the number of rows of kernels in 11 of the 13 families. The ear rows of each of these 11 families were classified into desirable and undesirable rows according as the plants in them approached the normal stability and vigor of open-fertilized corn. This classification was based upon a consideration of the culms, roots, leaves, chlorophyl, ears, and manner and time of dying.

Data on the relation of the number of kernel rows on the parent ears to the desirability or undesirability of the ear rows grown from them are shown by families in Table XIV. The last column in this table shows the differences between the average number of kernel rows on the parent ears producing desirable and undesirable ear rows in each of the 11 families. The parent ears producing desirable ear rows had fewer kernel rows than those producing undesirable ones in 9 of the 11 families, and averaged 1.52 of a row less in all of the rows.

If the future breeding stocks are selected on the basis of the most vigorous and productive ear rows, it is evident that the tendency will be to reduce the number of kernel rows on the ears in the families with higher numbers until the numbers in all families are about the same level. This is in agreement with the change in the number of rows in the Cuban Yellow and Boone County White varieties which showed the effects of this tendency during four years and three years of selection, respectively.

TABLE XIV.—*Data on the relation of number of rows of kernels on the parent ear to desirability or undesirability of plants in the ear rows from them in 11 self-fertilized families of Garrick at Baton Rouge, La., in 1923* [a]

Family No.	Kernel rows on the 1917 selfed seed ears	Classification of the 1923 ear rows	Parent ears with the number of kernel rows stated producing ear rows classed as desirable and undesirable							Total ear rows	Kernel rows on parent ears (average)	Difference between average number of kernel rows on parent ears of desirable and undesirable ear rows
			8	10	12	14	16	18	20			
32	10	Desirable	1	1	----	----	----	----	----	2	9.00	−0.14
		Undesirable	4	3	----	----	----	----	----	7	8.86	
57	10	Desirable	6	1	1	----	----	----	----	8	8.75	3.65
		Undesirable	----	----	4	1	----	----	----	5	12.40	
38	10	Desirable	----	2	----	----	----	----	----	2	10.00	1.33
		Undesirable	----	2	----	1	----	----	----	3	11.33	
49	12	Desirable	----	1	1	----	----	----	----	2	11.00	1.67
		Undesirable	----	----	2	1	----	----	----	3	12.67	
54	12	Desirable	----	1	4	----	1	----	----	6	12.33	.07
		Undesirable	----	1	2	2	----	----	----	5	12.40	
79	14	Desirable	----	1	----	2	3	----	----	6	14.33	−1.33
		Undesirable	----	----	1	1	----	----	----	2	13.00	
86	14	Desirable	----	----	1	5	----	1	----	7	14.29	.04
		Undesirable	----	----	3	1	1	----	1	6	14.33	
90	16	Desirable	----	----	----	2	----	----	----	2	14.00	2.00
		Undesirable	----	----	----	1	1	1	----	3	16.00	
93	16	Desirable	----	----	----	1	----	----	1	2	17.00	3.00
		Undesirable	----	----	----	----	----	----	1	1	20.00	
99	16	Desirable	----	----	----	2	----	----	----	2	14.00	2.40
		Undesirable	----	----	1	----	1	3	----	5	16.40	
107	18	Desirable	----	----	1	----	1	----	----	2	14.00	4.00
		Undesirable	----	----	----	----	1	1	1	3	18.00	
All families.		Desirable	----	----	----	----	----	----	----	41	12.61	1.52
		Undesirable	----	----	----	----	----	----	----	43	14.13	

[a] The data were obtained in cooperation with the Louisiana Agricultural Experiment Station.

DISCUSSION

Corn breeding probably has received more attention in this country than any other problem of crop improvement. If it were dependent only upon the selection of strains with constructive characters, such as size of ear, it would hardly be a serious problem. Yet this vast experience has created among the thoughtful only uncertainty and caution in making general recommendations. The possibilities of selection within self-fertilized lines as a means of corn improvement are being investigated extensively. The problem is not an easy one, however, and it is impossible to predict what the future may bring in the way of a practical application of this principle. In any event, the bulk of the seed corn planted in the United States will be obtained by mass selection for some time to come, and it therefore is important to know what characters, if any, can be used safely as a guide in such selection.

The experiments with open-fertilized seed reported give further evidence that seed ears with larger numbers of kernel rows and smaller and more angular kernels tend to produce lower yields. This was true in some of the experiments in spite of a higher yield per bearing plant because the seed from the many-rowed ears produced more barren plants. The data from the experiments with self-fertilized lines indicate that the larger proportion of weak and barren plants in strains with many-rowed ears was due to a tendency for such strains to have more deleterious characters.

Selection for large ears frequently has resulted in ears with larger numbers of kernel rows, and it seems probable that the benefits accruing from increased size of ear may have been nullified for the reasons already noted. The diameter of ear and the number of rows of kernels are correlated, though how much of this relation is unavoidable is unknown. Size of ear is dependent upon length as well as diameter, however, and the number of kernel rows need not be increased in obtaining longer ears. It therefore seems that selection on the basis of total length of ear (or ears) per plant, together with as much diameter as may be had without too many rows of kernels, may be recommended as satisfying the size requirements without increasing the ill effects that apparently are associated with a larger number of kernel rows. It is probable that the maximum length of total ear per plant can be obtained by having more than one ear on a plant. Whether this is necessary to obtain the maximum yield, however, or whether plants bearing one long ear may be equally productive under some conditions, remains to be determined.

SUMMARY

Experiments are reported in which prolific varieties were more productive as a class than comparable nonprolific varieties. The ears in the prolific varieties had fewer rows of kernels and smaller diameters and the kernels were less angular than in the nonprolific varieties.

In comparisons between groups of ears, both within prolific and nonprolific varieties, and within several F_1 crosses, those with fewer kernel rows, larger kernels, or less angular kernels were more productive. In the comparisons, both between and within varieties, the lots with fewer kernel rows produced fewer barren plants and, in some cases, made the larger total yield in spite of a smaller yield per bearing plant as measured by the yield index.

Selection for different numbers of kernel rows in selfed lines resulted in an essentially steady increase during five generations in the degree of conformity to the parental number. The rate of this increase was greatest in the lines with the fewest rows and decreased as the number of rows increased. Selection also resulted incidentally in other differences. Thus, the lines having smaller numbers of kernel rows had a greater length of ear per plant, ears with a smaller diameter, and kernels more rounded and with less indentation than the lines having larger numbers of kernel rows. Finally, the fewer-rowed lines were more resistant to corn smut, had fewer plants with heritable deleterious characters, and were more vigorous and productive in general.

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